

**REMARKS**

Claims 20 to 24 are pending in the present application. The following remarks and supporting documents are presented in support of the patentability of the pending claims.

**Rejection under 35 U.S.C. § 102(b)**

In the Office Action, claims 20-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Nakagawa et al (Cancer Research, 1988, Vol.48, pp.2096-2100) as evidenced by the abstract of Zabel et al (Histology and Histopathology, 1997, Vol.12, pp.283-289). Applicant respectfully traverse the rejection and submits that claims 20-22 are patentable within the meaning of 35 U.S.C. § 102(b). Reconsideration and withdrawal of the rejection are respectfully solicited.

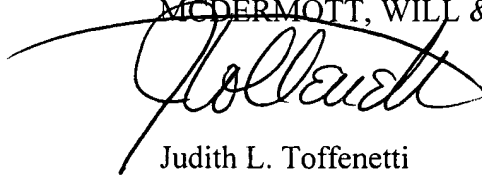
The present invention is directed to methods for inducing the re-expression of a previously silenced gene encoding the human sodium/iodide symporter in a human thyroid carcinoma cell by administering a compound selected from 5-azacytidine, sodium butyrate, dimethylsulfoxide, S-adenosyl-1,8-diamino-3-thio-octane, and phenylacetate. Applicant's studies have shown that these demethylating agents or differentiating agents can restore transcription of a previously inhibited iodide transporter gene, thus restoring iodide transport. Moreover, applicant's studies have demonstrated restoration of gene expression in a variety of human thyroid carcinoma cells, for example in follicular carcinoma cells. Therefore, the claimed invention provided a method for treating thyroid cancer cells which have lost the ability to transport iodine and which have an inactive thyroid specific response element. Applicants have shown that administration of a demethylating agent or differentiating agent to the cells results in expression of the therapeutic response element and uptake of iodine.

In contrast, Nakagawa et al describes that the butyrate treatment induces cultured human medullary thyroid carcinoma cells to acquire *in vitro* properties consistent with the differentiated phenotype of the mature thyroid cell. As mentioned by the Examiner, the TT cell is a parafollicular cell line. Medullary thyroid carcinoma derives from these parafollicular cells, notable for secreting calcitonin and other substances, and constitutes 5% to 10% of thyroid malignancies (see attached documents). However, parafollicular, or C, cells, lie between the follicles of the thyroid gland. These cells do not have the same embryological origin as do the thyroid follicular cells. A parafollicular cell does not need an active gene for sodium/iodide symporters since in this cells there is no active transport of iodide, since in this cells there is no synthesis of thyroid hormones. Therefore, medullary thyroid cancer is untreatable by the uptake of iodine. There is no inducible re-expression of a previously silenced gene encoding the human sodium/iodide symporter. Therefore, the human thyroid carcinoma cells according to the invention differ from the cells used in the cited documents. They are different cell types.

Accordingly, it is submitted that Nakagawa et al does not negate the patentability of the presently claimed invention. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) are respectfully requested.

Respectfully submitted,

MCDERMOTT, WILL & EMERY



Judith L. Toffenetti  
Registration No. 39,048

600 13th Street, N.W., Suite 1200  
Washington, DC 20005-3096  
Telephone: (202) 756-8000  
Facsimile: (202) 756-8087  
**Date: September 16, 2004**

# Advances and Controversies in the Diagnosis and Management of Medullary Thyroid Carcinoma

Hassan M. Heshmati, MD, Hossein Gharib, MD, Jon A. van Heerden, MD, Rochester, Minnesota, Glen W. Sizemore, MD, Maywood, Illinois

Recent advances in the diagnosis and treatment of medullary thyroid carcinoma (MTC) have been significant, but some issues remain controversial. MTC may occur either as a hereditary or a nonhereditary entity. Hereditary MTC can occur either alone—familial MTC (FMTC)—or as the thyroid manifestation of multiple endocrine neoplasia type 2 (MEN 2) syndromes (MEN 2A and MEN 2B). These hereditary disorders are due to germline mutations in the RET proto-oncogene. Early diagnosis and treatment considerably improve the prognosis in patients with MTC. Genetic testing can identify almost all affected individuals with hereditary disease and permits early thyroidectomy in gene carriers. Plasma CT is an excellent marker for postoperative follow-up. Imaging studies help delineate recurrent or metastatic lesions. Treatment of recurrent or metastatic disease is primarily surgical, including either palliative or microdissective surgery. Radiation therapy is reserved for skeletal metastasis or nonresectable metastatic MTC. Efficacy of current chemotherapy programs is not well established. Overall, the 10-year survival rates are approximately 65%. *Am J Med.* 1997;103:60–69. © 1997 by Excerpta Medica, Inc.

Medullary thyroid carcinoma (MTC), a rare thyroid malignancy originating from the parafollicular C cells, was first described by Hazard et al in 1959.<sup>1</sup> It represents less than 10% of all thyroid cancers and may occur either as a hereditary or a nonhereditary entity.<sup>2–4</sup> Hereditary MTC can occur either alone—familial MTC (FMTC)—or as the thyroid manifestation of multiple endocrine neoplasia

type 2 (MEN 2) syndromes (MEN 2A and MEN 2B). These hereditary disorders are due to germline mutations in the RET proto-oncogene.<sup>5–12</sup> Early diagnosis and treatment significantly improve the outcome of patients with MTC. Tests based on the detection of FMTC and MEN 2 mutations in genomic DNA have made possible the detection of asymptomatic gene carriers of hereditary MTC in at-risk family members.<sup>7,13,14</sup> This review describes recent advances in the diagnosis of MTC and discusses current controversies in management.

## CLASSIFICATION AND CLINICAL PRESENTATION

Nonhereditary or sporadic MTC is the most common type of MTC, accounting for approximately 80% of all cases at initial presentation. The remaining 20% comprise hereditary MTC including MEN 2A (the most common), MEN 2B, FMTC (the least common), and other hereditary MTCs (Table I).

MEN 2 was first described by Sipple in 1961.<sup>15</sup> MEN 2A is characterized by MTC, pheochromocytoma and hyperparathyroidism.<sup>16</sup> Three phenotypic subtypes have been reported.<sup>17</sup> MEN 2A(1) corresponds to kindreds with all three components. MEN 2A(2) includes kindreds with MTC and pheochromocytoma, without hyperparathyroidism. MEN 2A(3) relates to kindreds with MTC and hyperparathyroidism, without pheochromocytoma. A variant of MEN 2A, associated with pruritic skin lesions known as cutaneous lichen amyloidosis (CLA), has also been described in a few kindreds.<sup>18</sup> MEN 2B accounts for about 18% of patients with hereditary MTC and is characterized by MTC, pheochromocytoma, ganglioneuromatosis, and a marfanoid body habitus.<sup>16</sup> A variant of MEN 2B, with MTC and prominent corneal nerves without typical ganglioneuromatosis, was also described in a kindred.<sup>19</sup> FMTC includes kindreds with at least 4 members with MTC, without other components of MEN 2A or MEN 2B. Other hereditary MTCs correspond to kindreds with MTC in 2 or 3 members, without pheochromocytoma or hyperparathyroidism.

In sporadic MTC, the tumor is usually unifocal and discovered in the fifth or sixth decade of life. The clinical presentation includes a thyroid nodule or mass, cervical lymphadenopathy or other local cervical symptoms, and rarely diarrhea, flushing, Cush-

From the Division of Endocrinology/Metabolism and Internal Medicine (HMH, HG), and the Department of Surgery (JAvH), Mayo Clinic and Mayo Foundation, Rochester, Minnesota; and the Division of Endocrinology/Metabolism (GWS), Loyola University, Stritch School of Medicine, Maywood, Illinois.

Requests for reprints should be addressed to Hossein Gharib, MD, Division of Endocrinology/Metabolism and Internal Medicine, Mayo Clinic and Mayo Foundation, 200 First Street SW, Rochester, Minnesota 55905.

Manuscript submitted September 6, 1996 and accepted in revised form March 4, 1997.

BEST AVAILABLE COPY

**TABLE 1**  
Types, Frequency, and Clinical Presentation  
of MTC Syndromes

Phenotypes	Frequency	Clinical Presentation
Sporadic MTC	80%	MTC
Men 2A:		
MEN 2A(1)	4%	MTC, pheochromocytoma, hyperparathyroidism
MEN 2A(2)	4%	MTC, pheochromocytoma
MEN 2A(3)	1%	MTC, hyperparathyroidism
MEN 2B	3%	MTC, pheochromocytoma, ganglioneuromatosis, marfanoid habitus
FMTC	1%	MTC (in at least 4 patients)
Other FMTC	7%	MTC (in 2 or 3 patients)

MTC = medullary thyroid carcinoma; MEN 2A = multiple endocrine neoplasia type 2A; MEN 2B = multiple endocrine neoplasia type 2B; FMTC = familial MTC.

ing's syndrome due to chronic ectopic adrenocorticotrophic hormone (ACTH) production, and metastatic disease. In hereditary MTC, the MTC is usually multifocal and bilateral. Although it is difficult to determine the age of onset of the MTC with accuracy, it is thought that the disease develops much earlier in MEN 2B (where metastasis has occurred by age 4 years), whereas in MEN 2A and FMTC, the onset as well as metastasis occur later. The majority of hereditary patients are asymptomatic at discovery, because of detection by genetic or biochemical screening tests in the early stages of the disease.

Approximately 30% to 50% of patients with MEN 2 develop pheochromocytoma(s). The adrenomedullary disease is usually multicentric and bilateral, often detected after the onset of MTC, and malignant in less than 10% of patients.<sup>20,21</sup> Clinical manifestations of pheochromocytoma are usually subtle. Early symptoms include palpitations and nervousness; approximately 50% of patients may exhibit hypertension, which may be either paroxysmal or sustained. Sudden death may rarely occur as a result of a hypertensive crisis.<sup>20,21</sup> Thus, pheochromocytomas must be ruled out prior to operations and pregnancies in these patients, as they may induce a hypertensive crisis.

Hyperparathyroidism occurs in approximately 10% to 20% of MEN 2A patients. The disease usually involves multiple parathyroid glands and appears to be more occult than its sporadic counterpart.<sup>22</sup>

MEN 2B is characterized by its unique phenotypic appearance including diffuse ganglioneuromatosis involving the lips, tongue, eye, and gastrointestinal tract (Figure 1). The characteristic facies are generally recognizable in early childhood. The gastrointestinal involvement may cause intermittent constipation and diarrhea, abdominal pain, megacolon, and occasionally obstruction. Another phenotypic



**Figure 1.** Characteristic facies in a girl with multiple endocrine neoplasia type 2B. Features include elongated face, thick lumpy lips caused by mucosal neuromas, and thick eyelids with marked eversion.

feature of MEN 2B is a marfanoid habitus with long, thin extremities and fingers, hyperextensible joints, and epiphyseal abnormalities. However, these patients do not have the cardiac manifestations of Marfan's syndrome.

## BIOCHEMISTRY

MTC tumor cells produce a variety of substances. These include calcitonin (CT), CT gene-related peptide (CGRP), carcinoembryonic antigen (CEA), amyloid, somatostatin, ACTH, vasoactive intestinal peptide, prostaglandins, serotonin, substance P, histaminase, and melanin. CT is the main biochemical marker used for detection and postoperative management of patients with MTC. Basal plasma CT and CEA levels are elevated in the majority of patients with MTC. Plasma CGRP levels are elevated mainly in patients with large tumors or metastasis.

Pheochromocytoma secretes catecholamines (epinephrine, norepinephrine, dopamine). In some patients, the initial abnormality may be a minimal increase in urinary epinephrine or an increase in the ratio of urinary epinephrine to norepinephrine.

TABLE II

## Recognized Genetic Abnormalities in MTC Syndromes

Phenotypes	RET Mutations	Exons	Codons	Amino Acid Changes*
Sporadic MTC	Somatic (>20%)	13	768	Glu → Asp
		16	918	Met → Thr
MEN 2A	Germline (95%)	10	609	Cys → Tyr
		10	611	Cys → Tyr
		10	618	Cys → Ser
		10	620	Cys → Arg
		11	634	Cys → Arg
MEN 2B	Germline (94%)	16	918	Met → Thr
FMTC	Germline (87%)	10	609	Cys → Tyr
		10	611	Cys → Tyr
		10	618	Cys → Ser
		10	620	Cys → Ser
		11	634	Cys → Tyr
		13	768	Glu → Asp
		14	804	Val → Leu

MTC = medullary thyroid carcinoma; MEN 2A = multiple endocrine neoplasia type 2A; MEN 2B = multiple endocrine neoplasia type 2B; FMTC = familial MTC.  
 \* Examples correspond to the most common (or exclusive) amino acid substitutions for each codon.

The parathyroid disease, with an absolute or inappropriate increase in serum parathyroid hormone (PTH) level, is associated, in its typical form, with hypercalcemia and hypophosphatemia.

## GENETICS

MEN 2A, MEN 2B, and FMTC are autosomal dominant disorders. These are due to germline mutations in the RET proto-oncogene, located in the proximal region of the long arm of chromosome 10, band q11.2, which codes for a receptor-like tyrosine kinase (Table II).<sup>5-12</sup> The ligand for RET was recently identified.<sup>23</sup> RET is a functional receptor for glial-cell-line-derived neurotrophic factor (GDNF), a distant member of the transforming growth factor- $\beta$  superfamily. GDNF-induced activation of RET is mediated by GDNF receptor- $\alpha$ , a novel cell surface receptor for GDNF.<sup>24</sup> GDNF, in addition to its potential role in the differentiation and survival of central nervous system neurons, has profound effects on kidney organogenesis and the development of the peripheral nervous system. Mutations in MEN 2A, MEN 2B and FMTC convert RET into a dominant transforming gene with oncogenic activity.<sup>25</sup>

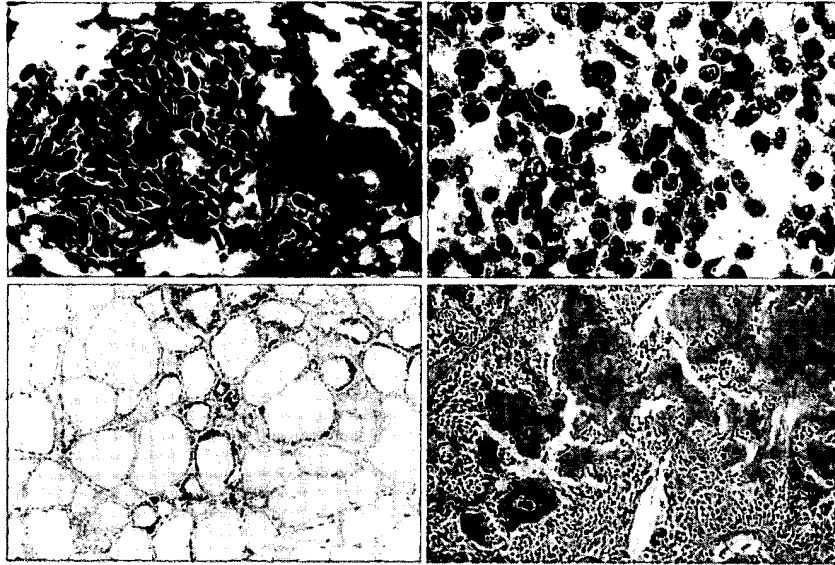
In FMTC and MEN 2A, the earliest recognized mutations affected 1 of 5 cysteine residues in exons 10 (codons 609, 611, 618 and 620) or 11 (codon 634), in the extracellular domain of the RET proto-oncogene.<sup>5-7</sup> These mutations may affect ligand binding of the RET protein. New germline mutations are very uncommon in MEN 2A and FMTC.<sup>26</sup> Recently, additional germline mutations have been reported in FMTC, for the glutamic acid residue in exon 13 (codon 768)<sup>27,28</sup> and valine residue in exon 14 (codon 804),<sup>27</sup> in the intracellular domain of the RET proto-oncogene. The main affected codon in MEN 2A kin-

dreds is codon 634 and the most common mutation in this codon is the Cys → Arg mutation. In FMTC, the main affected codon is codon 618.

In MEN 2A, a few studies have reported a correlation between genotype and phenotype. Patients with codon 634 mutations have a higher frequency of pheochromocytoma and hyperparathyroidism than those with other mutations.<sup>5,6</sup> With codon 634 mutations, subjects with the subtype Cys → Arg mutation have even a higher frequency of hyperparathyroidism than those with other codon 634 subtype mutations.<sup>5</sup>

The basis of the tissue specificity for these RET mutations in causing neoplasia is unexplained. Some authors suggest that a threshold effect may exist, with thyroid C cells being the most sensitive and parathyroid cells the least sensitive to the altered RET protein. Adrenal cells would have an intermediate sensitivity to the RET mutation.<sup>5,29</sup> Of particular interest is the phenotypic difference between MEN 2A and FMTC despite sometimes having identical RET mutations. The occurrence of distinct clinical phenotypes in patients sharing identical predisposing mutations could be explained in part by the failure to diagnose the more occult form of parathyroid and adrenomedullary diseases in these "FMTC" kindreds because of possible insensitivity of our diagnostic tests for these components, or by the influence of a modifier gene.

In MEN 2B, the mutation so far identified involves a methionine residue in exon 16 (codon 918), in the intracellular domain of the RET proto-oncogene.<sup>8,9</sup> This mutation may alter substrate specificity or catalytic properties of the RET protein. New germline mutations are frequently observed in these patients (50% of cases).<sup>30</sup> There is also evidence for genomic



**Figure 2.** Cytologic and histologic presentation of medullary thyroid carcinoma. Fine-needle aspiration biopsy shows spindle-shaped cytoplasmic processes and elongated nuclei; amyloid is also identified (**upper left**). Fine-needle aspiration biopsy specimen on immunoperoxidase staining for calcitonin is consistent with medullary thyroid carcinoma (**upper right**). Specimen from patient with familial medullary thyroid carcinoma shows foci of C-cell hyperplasia identified by positive immunostaining for calcitonin (**lower left**). Medullary thyroid carcinoma tumor containing large amounts of amyloid deposits in the stroma (**lower right**).

imprinting in MEN 2B as the affected offspring of transmitting males are predominantly female.<sup>30</sup>

Germline RET mutations are also reported in rare cases with presumed sporadic MTC.<sup>31</sup> These may correspond either to new germline mutations, or to MEN 2A or FMTC cases with rather poor or incomplete familial screening. A subset of patients with sporadic MTC (more than 20%) have somatic mutations in exons 13 or 16.<sup>8,28,31,32</sup>

## **PATHOLOGY**

Macroscopically, MTC is solid, firm and not encapsulated. Microscopically, it appears as nests of uniform round neoplastic cells. The most common patterns of growth are trabecular, alveolar, and spindle cells.<sup>33</sup> Multinucleation is common. Amyloid and calcifications are often present (**Figure 2**). In sporadic MTC, the tumor is usually unicentric, without a background of C-cell hyperplasia. Fewer than 20% of sporadic patients may have bilateral tumors and this is generally presumed to be an extension from the primary tumor. In familial syndromes, the MTC is usually bilateral, multicentric and most commonly present in the upper and middle thirds of each lobe. C-cell hyperplasia, the precursor of MTC, may be present early in life even at birth. The progression from hyperplasia to carcinoma may take years. Although C-cell hyperplasia is a characteristic histological feature of hereditary MTC, it may be observed occasionally in normal thyroid glands at autopsy and in some patients with various nonthyroidal neoplastic and nonneoplastic diseases. The diagnosis of

MTC can reliably be made on specimens obtained with fine-needle aspiration (FNA) biopsy (**Figure 2**). Amorphous amyloid may sometimes be identified in smears.

The adrenomedullary disease is usually multicentric, in a context of diffuse adrenal medullary hyperplasia, and bilateral—often asynchronously presenting pheochromocytomas.<sup>21</sup>

Hyperparathyroidism in MEN 2A is usually due to chief cell hyperplasia of multiple parathyroid glands (diffuse but unequal multigland hyperplasia). Parathyroid adenomas are less common. However, a recent study reports single adenoma in 54% of cases.<sup>22</sup>

## **SCREENING**

In kindreds with hereditary MTC, genetic screening is the easiest and the most cost-effective approach to detect affected patients.<sup>13,34–39</sup> Genetic testing can, and should, be performed very early in life (soon after birth). In a kindred with a known mutation, members predicted not to be gene carriers by RET mutation analysis do not require further genetic or biochemical testing; no tests need to be performed on their descendants. In contrast, subjects predicted to have inherited the predisposing gene should be considered at high risk of developing MTC (if not already present). The knowledge of the particular mutations in MEN 2A kindreds may also be helpful in the follow-up with biochemical testing for the detection of other components of the disease (early detection of pheochromocytoma or hyperparathyroidism in patients having codon 634 mutations).

When properly performed, genetic testing using RET analysis should have no false-negative or false-positive results; however, to avoid sample mix-up, some prefer repeating a negative or positive test. The possibility of false paternity should also be considered when interpreting the final results. In RET mutation-negative kindreds, linkage analysis may identify gene carriers. However, linkage analysis is not as accurate as RET mutation analysis because of errors due to recombinations.<sup>7</sup> Thus, primary relatives of MTC-affected patients with no identified RET mutation and with negative linkage analysis results, require regular follow-up by biochemical tests.

In sporadic MTC and hereditary MTC with negative genetic testing, measurement of basal and stimulated plasma CT is the preferred method for the diagnosis of MTC. Because some patients with occult MTC or C-cell hyperplasia have normal basal CT levels, provocative testing with pentagastrin or calcium (Ca) and pentagastrin have been used to increase detection rates. In comparing results of CT stimulation for this purpose, we and others have found pentagastrin to be the most effective CT secretory discriminator in patients with MTC.<sup>40,41</sup> Thus, pentagastrin stimulation is now almost universally used by most laboratories. The test can be performed in early childhood or even infancy. Plasma CT is measured before, and 1.5 and 5 minutes after the intravenous injection over 5 seconds of 0.5 µg/kg of body weight of pentagastrin contained in 5 mL of 0.9% NaCl as a bolus. We have described a more accurate and sensitive CT measurement using an extraction radioimmunoassay, with few false-positive or false-negative results.<sup>42</sup> The normal range for basal and stimulated CT levels varies between different laboratories; ours are illustrated in Table III. In kindreds with familial MTC and negative genetic testing, at-risk individuals with negative biochemical results should be tested annually until the age of 35 years because conversion from negative to positive CT stimulation test has been reported to occur in 95% by the fourth decade of life.<sup>41</sup> The pentagastrin test has several unpleasant small peptide side effects including metallic taste, esophageal spasm, nausea, flushing, warmth, and the urge to void. These symptoms are mild and transient, and usually resolve within 2 minutes. False-negative or false-positive pentagastrin stimulation tests may be observed in 5% to 18% of cases.<sup>7,43</sup> False-negative results most probably occur in patients who have not yet developed sufficient C-cell volume to be detected as abnormal. False-positive results may be observed in normal subjects with C-cell disease for unknown reasons but without MTC.

In MEN 2, adrenomedullary disease is sought by annual measurement of urinary metanephrines and

TABLE III

Differences in Basal and Pentagastrin-stimulated\* Plasma CT Levels in Normal Subjects

Sex	Basal CT (pg/mL)	Stimulated CT (pg/mL)
Male	0-19	≤110
Female	0-14	≤30

\* Pentagastrin (0.5 µg/kg of body weight) by a bolus injection with blood sampling at 0, 1.5, and 5 minutes (modified from Gharib et al, 1987). CT, calcitonin.

fractionated catecholamines (epinephrine, norepinephrine, dopamine), after the age of 6 years.<sup>20,37,41,44</sup> Elevated epinephrine or an elevated epinephrine/norepinephrine ratio is the most commonly observed pattern. No relationship exists between tumor size and catecholamine production.

We screen for hyperparathyroidism by measurement of serum Ca every 2 years. Once hypercalcemia is documented, serum intact PTH should be measured to confirm the diagnosis of hyperparathyroidism.<sup>37,41</sup>

## OTHER INVESTIGATIONS

The preoperative diagnosis of MTC can be further confirmed by FNA if a thyroid nodule and/or cervical lymphadenopathy is present. Chest x-ray, computed tomography, or magnetic resonance imaging (MRI) scans are also helpful to determine the presence of distant metastases.

When biochemical diagnosis of pheochromocytoma is established, the tumor should be localized by computed tomography or MRI scans; iodine-131 meta-iodobenzylguanidine (<sup>131</sup>I-MIBG) scintigraphy is seldom used but may be helpful if malignancy is suspected.

High-resolution small part sonography is sometimes used to differentiate parathyroid hyperplasia from solitary adenoma.

## TREATMENT

Because MTC is the main cause of death in patients with MEN 2 and FMTC, early diagnosis and treatment are mandatory. The aim should be to identify as many patients as early as possible before gross disease is evident, particularly disease that has extended beyond the confines of the thyroid gland. The prognosis varies greatly depending upon the extent of disease; ie, C-cell hyperplasia (best), gross disease confined to the thyroid, or extrathyroidal extension (worst). Ideally, as many patients as possible should be identified at the precancerous C-cell hyperplasia phase. Based on the results of genetic testing, subjects predicted to have inherited the predisposing gene should have thyroidectomy at an early age (between the ages of 5 and 10 years for MEN 2A

and FMTC, earlier for MEN 2B). In a recent study by Wells et al,<sup>13</sup> 6 patients from MEN 2A kindreds between ages 6 to 14 years who had RET mutations but normal stimulated CT levels, underwent prophylactic thyroidectomy; 3 had C-cell hyperplasia, 2 had microscopic MTC, and 1 had macroscopic MTC.

Total thyroidectomy is recommended for all patients with MTC (hereditary or nonhereditary), or those at risk of developing MTC,<sup>16,45,46</sup> since in familial MTC the tumor is multifocal and bilateral, and in sporadic MTC the incidence of bilateral disease is not insignificant (up to 20% of cases). Central compartment node dissection (nodes in the tracheoesophageal groove plus nodes above and below the thyroid isthmus) should routinely accompany total thyroidectomy.<sup>45-47</sup> If central nodes are negative, sampling of the lateral compartment nodes is not necessary. If central plus lateral nodes are positive, a modified neck dissection is indicated with preservation of the accessory spinal nerve, the sternomastoid muscle, and the internal jugular vein.<sup>16,46</sup> Nodal metastases are more common in patients with MEN 2B and sporadic disease, followed by patients with FMTC and MEN 2A. The study of 123 patients who underwent thyroidectomy for MTC showed the presence of cervical lymph node metastasis in 54%, 53%, and 24%, in sporadic MTC, MEN 2B, and MEN 2A, respectively.<sup>46</sup> We and others have shown that there is no prognostic advantage of a radical versus a modified radical neck dissection.<sup>16,45</sup>

Some authors express concern regarding the risk of complications of surgery (recurrent laryngeal nerve damage, hypoparathyroidism) in young children. In most series, including our own, the risk of permanent recurrent laryngeal nerve damage and permanent hypoparathyroidism is no greater than that occurring in the adult population.<sup>13,37,48</sup> Of these two possible complications, permanent hypoparathyroidism is, by far, the most troublesome and should be avoided at all costs, if possible. This can best be accomplished by meticulous preservation, in situ, of as many parathyroid glands as possible, and particularly visualization of all four glands when a total thyroidectomy is performed. If any parathyroid glands are to be sacrificed because of their anatomical location, immediate autotransplantation into the strap muscles or the sternocleidomastoid muscle in the neck should be performed, and the transplanted area marked with either a silver clip, or nonabsorbable suture. For patients electing not to undergo early thyroidectomy, periodic biochemical testing (CT determination) will determine the proper time for surgery, since abnormal CT levels mandate surgical treatment.<sup>38</sup>

In MTC patients, thyroid surgery should be undertaken only with the knowledge of catecholamine se-

cretion. If excessive catecholamine secretion is demonstrated, adrenal surgery should precede thyroidectomy. In our experience, adrenomedullary disease (pheochromocytomas or adrenal medullary hyperplasia) is uniformly bilateral. The pathology may vary from bilateral adrenomedullary hyperplasia to bilateral pheochromocytomas to unilateral pheochromocytomas that may be unifocal, or multifocal with contralateral hyperplasia. Our philosophy has been initial bilateral total adrenalectomy, a procedure that is extremely well tolerated with appropriate adrenal replacement therapy. Although there is some controversy regarding unilateral versus bilateral initial adrenalectomy, we have not embraced the philosophy of initial unilateral resection. Adrenal surgery must be preceded by appropriate alpha (dibenzylamine) and beta (propranolol) blockade for approximately 7 to 10 days, as well as volume repletion.

The majority of MEN 2A patients with hyperparathyroidism will have diffuse but unequal multiglandular hyperplasia. A small percentage (10% to 15%) might in fact have initial single gland disease. There is controversy regarding total parathyroidectomy with immediate autotransplantation of a portion of a single gland versus subtotal parathyroidectomy with preservation of a well vascularized portion of a single gland in situ. We have adopted the latter approach. This approach should only be performed after visualization of all 4 glands and after the decision is made as to which of the glands can most safely be preserved in situ. We aim to preserve approximately 60 to 80 mg of viable tissue, and this gland is carefully marked for later identification. In addition, it is our philosophy that all of these patients should undergo routine transcervical thymectomy, since at least 12% to 15% of MEN 2A patients will have a fifth gland located in the mediastinal thymus.

## POSTOPERATIVE MANAGEMENT

After total thyroidectomy, all patients should receive lifelong replacement therapy with levothyroxine (L-T<sub>4</sub>). The dose of L-T<sub>4</sub> should be adjusted by periodic serum thyrotropin (TSH) determinations. Serum TSH level should be maintained within the normal range; there is no need for TSH suppression in these patients. Follow-up of MTC is mainly based on basal (only if high) and stimulated plasma CT determinations. Sequential undetectable or normal CT concentrations usually indicate cure. High concentrations in the immediate postoperative period indicate persistent disease. Elevations that occur after a disease-free period suggest recurrent or metastatic disease. Patients with microcarcinoma (tumor  $\leq$  1 cm), and/or C-cell hyperplasia with normal postoperative basal CT concentrations, are considered cured. Patients with multicentric, larger tumors



should have determination of both basal and stimulated CT concentrations. More patients with MTC and lymph node metastasis will have persistent disease and elevated postoperative CT concentrations than those with less initial disease.<sup>13,47</sup>

Patients with bilateral adrenalectomy should receive lifelong replacement therapy with appropriate glucocorticoid and mineralocorticoid therapy. In patients with recurrent symptoms or signs of pheochromocytoma, repeat measurements of catecholamines should be undertaken to detect recurrent or metastatic pheochromocytoma. This is especially important if the patient has had unilateral adrenalectomy.

Postoperative hypoparathyroidism (which is rare) is best, and more safely, treated with 1,25-dihydroxyvitamin D and adequate Ca supplements in sufficient quantities to stop symptoms, produce low normal serum Ca concentrations and yet avoid hypercalciuria.

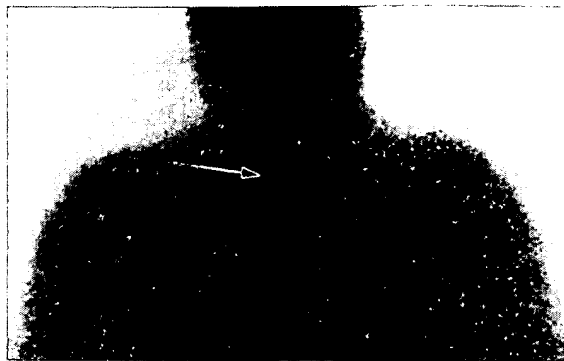
Patients who have not yet shown signs of pheochromocytoma or hyperparathyroidism should be screened at regular intervals, as detailed previously. Special attention should be paid to patients with codon 634 mutations, who are more prone to develop these components.

### PERSISTENT, RECURRENT, OR METASTATIC THYROID DISEASE

There is general agreement that high postoperative plasma CT concentrations always indicate persistent disease. When basal plasma CT levels are normal but persistent, or recurrent disease is suspected, stimulation testing should be performed. With the demonstration of either abnormal basal or stimulated CT levels, further investigations to localize MTC are indicated.

CEA is another tumor marker in patients with metastatic MTC. High values indicate residual disease. However, because CT is the more commonly elevated marker in this clinical situation, and CEA is not detected or increased in many patients with metastatic MTC, we use it only in unusual cases in which CEA is dominant to CT, particularly when cost-effectiveness is important.

The methods of localization and management of persistent disease remain controversial. The most frequent sites of metastatic disease are the nodes in the neck and superior mediastinum. Distant metastases are seen in the order of frequency in the liver, mediastinum, lung, and bone. Imaging studies useful in detecting disease include ultrasonography, chest x-ray, computed tomography or MRI, and radionuclide scanning using thallium-201 thallous chloride (<sup>201</sup>TlCl), technetium-99m pentavalent dimercaptosuccinic acid (<sup>99m</sup>Tc-DMSA) or indium-111 pentetreotide (<sup>111</sup>In-pentetreotide).<sup>4</sup>



**Figure 3.** Indium-111 pentetreotide scan in a patient with elevated postoperative serum calcitonin levels. The image shows abnormal uptake in the right neck on anterior 24-hour planar view. Neck exploration confirmed the presence of recurrent medullary thyroid carcinoma. Ultrasonography and computed tomography had failed to identify tumor.

In our experience, sonography is extremely sensitive in detecting persistent or recurrent malignancy in the thyroid bed and in neck lymph nodes. Ultrasonography has the advantage of identifying and permitting biopsy if suspicious lesions are seen. Computed tomography or MRI of the neck, mediastinum, and chest is also helpful in identifying metastatic deposits. However, these techniques are not very specific. <sup>201</sup>TlCl has been used successfully in detecting metastatic disease (92% of cases).<sup>49</sup> <sup>99m</sup>Tc-DMSA has high sensitivity but low specificity, and has found limited use in the postoperative management of patients with MTC (detection in 45% of cases).<sup>50</sup> The most recent and promising addition to these methods is somatostatin-receptor imaging. Scintigraphy with <sup>111</sup>In-pentetreotide has been used in a number of centers during the past 5 years.<sup>50-53</sup> Although the tumor site can be visualized in the majority of cases (65%) (**Figure 3**), this technique is expensive and is insensitive in detecting liver metastases. Other methods of investigating metastatic MTC include selective venous catheterization, radioimmunoscintigraphy with monoclonal antibodies (<sup>131</sup>I-anti-CEA), and radioactive MIBG (<sup>131</sup>I-MIBG). Selective venous catheterization, now seldom used, determines CT gradient, thus localizing the area where tumor cells have metastasized.<sup>54</sup> Abdelmoumene et al<sup>55</sup> have reported good success in localizing metastasis (92% of cases) in patients with suspected disease, with this laborious and expensive technique. Scintigraphy using anti-CEA antibodies can detect metastatic MTC in the majority of cases (86%).<sup>49</sup> <sup>131</sup>I-MIBG is taken up by MTC. It has been used occasionally to detect metastatic MTC,<sup>49,56</sup> but it is probably not as sensitive for detection of micrometastasis as selective venous catheterization with pentagastrin or radioimmuno-

scintigraphy with monoclonal antibodies (detection in 27% of cases).

Therapy of patients with metastatic MTC is primarily surgical.<sup>67</sup> In patients with well localized and isolated disease this is especially true. There are differing views about those with occult metastatic MTC (patients with high plasma CT without clinically apparent tumor). In a review of this problem, Pommier and Brennan<sup>2</sup> discussed two different surgical approaches. The first is an aggressive approach to localize and cure, first reported in 1986 by Tisell et al.<sup>68</sup> They reported success with careful, extensive lymph node dissection in 11 patients with persistent MTC. This "microdissection" technique normalized postoperative CT levels in 4 patients (36%), with a duration of follow-up ranging from 2 to 4.5 years. In a subsequent report of 40 patients with MTC, the same authors found that after the first operation, 63% of the patients, and at the last test (mean follow-up, 6 years), 30% of the patients, had undetectable basal CT levels.<sup>69</sup> However, 35 of 40 patients had progressively increasing stimulated plasma CT levels with time. The second approach is a more conservative, palliative treatment. In a publication by van Heerden et al,<sup>60</sup> studying 31 patients with persistent hypercalcitoninemia after apparent curative primary surgery for MTC, of 11 patients having reoperation based on clinical or radiological signs of recurrence, none had normal basal CT levels postoperatively. However, the overall 10-year survival rate was 86%, and only 2 patients died from MTC. Clearly, microdissection surgery takes longer, the surgical technique is more difficult, and complications are greater than conventional surgery. Therefore, on the basis of available data on the risk-benefit ratio and the excellent long-term MTC prognosis, our approach, similar to most experienced centers, is conservative surgery for recurrent MTC. However, this issue remains unresolved and, in our opinion, multicenter comparative studies would be beneficial.

Other treatments including external beam radiation, radionuclide (<sup>131</sup>I, <sup>131</sup>I-MIBG, <sup>111</sup>In-pentetreotide), chemotherapy, octreotide, and interferon have also been proposed.<sup>4</sup> The effectiveness of external radiotherapy remains controversial; recent reports suggesting no cures and only partial responses.<sup>61,62</sup> The potential benefits of radiotherapy must be weighed against its reported complications (cervical fibrosis, radiation tracheitis, chronic dysphagia, paraplegia). However, surgically unresectable lesions, for example, skeletal or perispinal cord metastasis, may be palliated by treatment with radiotherapy. <sup>131</sup>I has no value as an adjunct to surgery because the C cells do not concentrate iodine and survival rate is not affected with its use.<sup>63</sup> The selective uptake of <sup>131</sup>I-MIBG and <sup>111</sup>In-pentetreotide by

TABLE IV

## Prognostic Factors in MTC Syndromes

Factors	Good Prognosis	Poor Prognosis
RET mutations	Codons 609, 611, 618, 620, 634, 768, 804	Codon 918
Sex	Female	Male
Plasma CT	Low	High
Plasma CT/CGRP	High	Low
Plasma CEA	Low	High
Age at surgery	Young	Old
Surgical resection	Complete	Incomplete
Tumor size	Small	Large
DNA ploidy	Diploid	Nondiploid
CT immunoreactivity	High	Low
Amyloid staining	Positive	Negative
Extrathyroidal invasion	Absent	Present
Nodal metastasis	Absent	Present
Distant metastasis	Absent	Present

MTC = medullary thyroid carcinoma; CT = calcitonin; CGRP = calcitonin gene-related peptide; CEA = carcinoembryonic antigen; DNA = deoxyribonucleic acid.

MTC tumor cells has generated interest in their potential use for targeted radiotherapy for MTC.<sup>64,65</sup> The tumor responses have been variable. Experience with chemotherapeutic agents in the management of MTC is limited and controversial.<sup>66,67</sup> Different combinations including cyclophosphamide + vincristine + dacarbazine,<sup>68</sup> or bleomycin + adriamycin + platinum, or dacarbazine + 5-fluorouracil,<sup>69</sup> appear to have moderate activity (partial response in 43% to 60% of cases) and are well tolerated. Long-term treatment with octreotide has not significantly improved biochemical and morphological parameters in patients with MTC.<sup>70,71</sup> Octreotide therapy should be considered for symptomatic treatment of refractory diarrhea in MTC.<sup>72</sup> The use of recombinant interferon alpha-2a has also been suggested in a preliminary study.<sup>73</sup>

## COURSE AND PROGNOSTIC FACTORS

The clinical course of patients with MTC varies with tumor specifics as well as its associations. Overall, 10-year survival rates are approximately 65%. Patients with MEN 2B have the most aggressive tumors, with early development of metastases (neck, liver, mediastinum, lung, bone) and death from the tumor. The biologic virulence of MTC in MEN 2B is followed by sporadic MTC, MEN 2A, and FMTC. Numerous parameters have been proposed as prognostic factors in the outcome for patients with MTC (Table IV).<sup>74,75</sup> Age at onset, stage, and completeness of initial surgical resection are significant factors in determining the outcome of MTC. In a retrospective study of 202 patients comparing the prognosis of patients with sporadic MTC and those with familial

MTC, patients with familial MTC had a significantly higher survival rate than patients with sporadic MTC.<sup>61</sup> In this study, age at onset was the major prognostic factor. However, when patients were matched for age, gender, extent of disease, and treatment, the difference in survival disappeared.

## CONCLUSION

Early diagnosis and treatment considerably improve the prognosis in patients with MTC. Genetic testing can identify almost all affected individuals with hereditary disease and allow us to perform early thyroidectomy in gene carriers. Primary treatment is surgical, and early total thyroidectomy in experienced centers is urged, as it can be performed with negligible morbidity and mortality. Plasma CT is an excellent marker in postoperative follow-up. Imaging studies help delineate recurrent or metastatic lesions. The possible benefits of microdissection for recurrent disease require further comparative study. Radiation therapy is reserved for skeletal metastasis or nonresectable metastatic MTC. Chemotherapy regimens have a limited palliative role.

## REFERENCES

- Hazard JB, Hawk WA, Crile G Jr. Medullary (solid) carcinoma of the thyroid; a clinicopathologic entity. *J Clin Endocrinol Metab.* 1959;19:152-161.
- Pommier RF, Brennan MF. Medullary thyroid carcinoma. *Endocrinologist.* 1992;2:393-405.
- Gharib H, McConahey WM, Tieg RD, et al. Medullary thyroid carcinoma: clinicopathologic features and long-term follow-up of 65 patients treated during 1946 through 1970. *Mayo Clin Proc.* 1992;67:934-940.
- Marsh DJ, Learoyd DL, Robinson BG. Medullary thyroid carcinoma: recent advances and management update. *Thyroid.* 1995;5:407-424.
- Mulligan LM, Eng C, Healey CS, et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. *Nat Genet.* 1994;6:70-74.
- Schuffenecker I, Billaud M, Calender A, et al. RET proto-oncogene mutations in French MEN 2A and FMTC families. *Hum Mol Genet.* 1994;3:1939-1943.
- Tsai MS, Ledger GA, Khosla S, et al. Identification of multiple endocrine neoplasia, type 2 gene carriers using linkage analysis and analysis of the RET proto-oncogene. *J Clin Endocrinol Metab.* 1994;78:1261-1264.
- Hofstra RMW, Landsvater RM, Ceccherini I, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature.* 1994;367:375-376.
- Carlson KM, Dou S, Chi D, et al. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. *Proc Natl Acad Sci USA.* 1994;91:1579-1583.
- Mulligan LM, Ponder BAJ. Genetic basis of endocrine disease: multiple endocrine neoplasia type 2. *J Clin Endocrinol Metab.* 1995;80:1989-1995.
- Goodfellow PJ, Wells SA Jr. RET gene and its implications for cancer. *J Natl Cancer Inst.* 1995;87:1515-1523.
- Donis-Keller H. The RET proto-oncogene and cancer. *J Intern Med.* 1995;238:319-325.
- Wells SA Jr, Chi DD, Toshima K, et al. Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. *Ann Surg.* 1994;220:237-247.
- Lips CJM, Landsvater RM, Höppener JWM, et al. From medical history and biochemical tests to presymptomatic treatment in a large MEN 2A family. *J Intern Med.* 1995;238:347-356.
- Sipple JH. The association of pheochromocytoma with carcinoma of the thyroid gland. *Am J Med.* 1961;31:163-166.
- Chong GC, Beahrs OH, Sizemore GW, Woolner LH. Medullary carcinoma of the thyroid gland. *Cancer.* 1975;35:695-704.
- Mulligan LM, Marsh DJ, Robinson BG, et al. Genotype-phenotype correlation in multiple endocrine neoplasia type 2: report of the International RET mutation Consortium. *J Intern Med.* 1995;238:343-346.
- Gagel RF, Levy ML, Donovan DT, et al. Multiple endocrine neoplasia type 2a associated with cutaneous lichen amyloidosis. *Ann Intern Med.* 1989;111:802-806.
- Kane LA, Tsai MS, Gharib H, et al. Familial medullary thyroid cancer and prominent corneal nerves: clinical and genetic analysis. *J Clin Endocrinol Metab.* 1995;80:289-293.
- Casanova S, Rosenberg-Bourgin M, Farkas D, et al. Pheochromocytoma in multiple endocrine neoplasia type 2A: survey of 100 cases. *Clin Endocrinol (Oxf).* 1993;38:531-537.
- Modigliani E, Vasen HM, Raue K, et al. Pheochromocytoma in multiple endocrine neoplasia type 2: European study. *J Intern Med.* 1995;238:363-367.
- Raue F, Kraimps JL, Dralle H, et al. Primary hyperparathyroidism in multiple endocrine neoplasia type 2A. *J Intern Med.* 1995;238:369-373.
- Durbec P, Marcos-Gutierrez CV, Kilkenny C, et al. GDNF signalling through the Ret receptor tyrosine kinase. *Nature.* 1996;381:789-793.
- Jing S, Wen D, Yu Y, et al. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. *Cell.* 1996;85:1113-1124.
- Santoro M, Carlomagno F, Romano A, et al. Activation of RET as a dominant transforming gene by germline mutations of MEN 2A and MEN 2B. *Science.* 1995;267:381-383.
- Zedenius J, Wallin G, Hamberger B, et al. Somatic and MEN 2A de novo mutations identified in the RET proto-oncogene by screening of sporadic MTC:s. *Hum Mol Genet.* 1994;3:1259-1262.
- Bolino A, Schuffenecker I, Luo Y, et al. RET mutations in exons 13 and 14 of FMTC patients. *Oncogene.* 1995;10:2415-2419.
- Eng C, Smith DP, Mulligan LM, et al. A novel point mutation in the tyrosine kinase domain of the RET proto-oncogene in sporadic medullary thyroid carcinoma and in a family with FMTC. *Oncogene.* 1995;10:509-513.
- Komminoth P, Kunz E, Hiort O, et al. Detection of RET proto-oncogene point mutations in paraffin-embedded pheochromocytoma specimens by non-radioactive single-strand conformation polymorphism analysis and direct sequencing. *Am J Pathol.* 1994;145:922-929.
- Carlson KM, Bracamontes J, Jackson CE, et al. Parent-of-origin effects in multiple endocrine neoplasia type 2B. *Am J Hum Genet.* 1994;55:1076-1082.
- Eng C, Mulligan LM, Smith DP, et al. Mutation of the RET protooncogene in sporadic medullary thyroid carcinoma. *Genes Chromosom Cancer.* 1995;12:209-212.
- Maeda S, Namba H, Takamura N, et al. A single missense mutation in codon 918 of the RET proto-oncogene in sporadic medullary thyroid carcinomas. *Endocr J.* 1995;42:245-250.
- Papotti M, Sambataro D, Pecchioni C, Bussolati G. The pathology of medullary carcinoma of the thyroid: review of the literature and personal experience on 62 cases. *Endocr Pathol.* 1996;7:1-20.
- Ponder BAJ. Genetic screening for multiple endocrine neoplasia type 2. *Exp Clin Endocrinol.* 1993;101:53-56.
- McMahon R, Mulligan LM, Healey CS, et al. Direct, non-radioactive detection of mutations in multiple endocrine neoplasia type 2A families. *Hum Mol Genet.* 1994;3:643-646.
- Xue F, Yu H, Maurer LH, et al. Germline RET mutations in MEN 2A and FMTC and their detection by simple DNA diagnostic tests. *Hum Mol Genet.* 1994;3:635-638.
- Thomas PM, Gagel RF. Advances in genetic screening for multiple endocrine neoplasia type 2 and the implications for management of children at risk. *Endocrinologist.* 1994;4:140-146.
- Ledger GA, Khosla S, Lindor NM, et al. Genetic testing in the diagnosis and management of multiple endocrine neoplasia type II. *Ann Intern Med.* 1995;122:118-124.
- Gagel RF, Cote GJ, Martins Bugalho MJG, et al. Clinical use of molecular information in the management of multiple endocrine neoplasia type 2A. *J Intern Med.* 1995;238:333-341.

40. Gharib H, Kao PC, Heath H III. Determination of silica-purified plasma calcitonin for the detection and management of medullary thyroid carcinoma: comparison of two provocative tests. *Mayo Clin Proc.* 1987;62:373-378.
41. Calmettes C, Ponder BAJ, Fischer JA, Raue F, and the members of the European Community Concerted Action: Medullary Thyroid Carcinoma. Early diagnosis of the multiple endocrine neoplasia type 2 syndrome: consensus statement. *Eur J Clin Invest.* 1992;22:755-760.
42. Kao PC, Gharib H. Clinical performance of an extraction calcitonin radioimmunoassay. *Mayo Clin Proc.* 1993;68:1165-1170.
43. Lips CJM, Landsvater RM, Höppener JWM, et al. Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. *NEJM.* 1994;331:828-835.
44. Easton DF, Ponder MA, Cummings T, et al. The clinical and screening age-at-onset distribution for the MEN2 syndrome. *Am J Hum Genet.* 1989;44:208-215.
45. Russell CF, van Heerden JA, Sizemore GW, et al. The surgical management of medullary thyroid carcinoma. *Ann Surg.* 1983;197:42-48.
46. Block MA. Surgical treatment of medullary carcinoma of the thyroid. *Otolaryngol Clin North Am.* 1990;23:453-473.
47. Dralle H, Scheumann GFW, Proye C, et al. The value of lymph node dissection in hereditary medullary thyroid carcinoma: a retrospective, European, multicentre study. *J Intern Med.* 1995;238:357-361.
48. Telander RL, Zimmerman D, Sizemore GW, et al. Medullary carcinoma in children. Results of early detection and surgery. *Arch Surg.* 1989;124:841-843.
49. Hoefnagel CA, Delprat CC, Zanin D, van der Schoot JB. New radionuclide tracers for the diagnosis and therapy of medullary thyroid carcinoma. *Clin Nucl Med.* 1988;13:159-165.
50. Bernà L, Cabezas R, Mora J, et al.  $^{111}\text{In}$ -octreotide and  $^{99\text{m}}\text{Tc}$ (V)-dimercaptosuccinic acid studies in the imaging of recurrent medullary thyroid carcinoma. *J Endocrinol.* 1995;144:339-345.
51. Kwekkeboom DJ, Reubi JC, Lamberts SWJ, et al. In vivo somatostatin receptor imaging in medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 1993;76:1413-1417.
52. Ahlman H, Tisell LE, Wängberg B, et al. Somatostatin receptors on neuroendocrine tumors: a way to intraoperative diagnosis and localization. *Yale J Biol Med.* 1994;67:215-221.
53. Krausz Y, Ish-Shalom S, Dejong RBJ, et al. Somatostatin-receptor imaging of medullary thyroid carcinoma. *Clin Nucl Med.* 1994;19:416-421.
54. Moley JF, Wells SA Jr. Medullary thyroid cancer: management of the patient with persistent hypercalcitoninemia. *Adv Endocrinol Metab.* 1994;5:25-45.
55. Abdelmoumene N, Schlumberger M, Gardet P, et al. Selective venous sampling catheterisation for localisation of persisting medullary thyroid carcinoma. *Br J Cancer.* 1994;69:1141-1144.
56. Sone T, Fukunaga M, Otsuka N, et al. Metastatic medullary thyroid cancer: localization with iodine-131 metaiodobenzylguanidine. *J Nucl Med.* 1985;26:604-608.
57. Buhr HJ, Kallinowski F, Herfarth C. Surgical strategies and methods for the treatment of metastasizing medullary thyroid carcinoma. *Recent Results Cancer Res.* 1992;125:147-165.
58. Tisell LE, Hansson G, Jansson S, Salander H. Reoperation in the treatment of asymptomatic metastasizing medullary thyroid carcinoma. *Surgery.* 1986;99:60-66.
59. Tisell LE, Dilley WG, Wells SA Jr. Progression of postoperative residual medullary thyroid carcinoma as monitored by plasma calcitonin levels. *Surgery.* 1996;119:34-39.
60. van Heerden JA, Grant CS, Gharib H, et al. Long-term course of patients with persistent hypercalcitoninemia after apparent curative primary surgery for medullary thyroid carcinoma. *Ann Surg.* 1990;212:395-401.
61. Samaan NA, Schultz PN, Hickey RC. Medullary thyroid carcinoma: prognosis of familial versus sporadic disease and the role of radiotherapy. *J Clin Endocrinol Metab.* 1988;67:801-805.
62. Nguyen TD, Chassard JL, Lagarde P, et al. Results of postoperative radiation therapy in medullary carcinoma of the thyroid: a retrospective study by the French Federation of Cancer Institutes—The Radiotherapy Cooperative Group. *Radiother Oncol.* 1992;23:1-5.
63. Saad MF, Guido JJ, Samaan NA. Radioactive iodine in the treatment of medullary carcinoma of the thyroid. *J Clin Endocrinol Metab.* 1983;57:124-128.
64. Forssell-Aronsson E, Fjälling M, Nilsson O, et al. Indium-111 activity concentration in tissue samples after intravenous injection of indium-111-DTPA-D-Phe-1-octreotide. *J Nucl Med.* 1995;36:7-12.
65. Wiseman GA, Kvols LK. Therapy of neuroendocrine tumors with radiolabeled MIBG and somatostatin analogues. *Semin Nucl Med.* 1995;25:272-278.
66. De Besi P, Busnardo B, Toso S, et al. Combined chemotherapy with bleomycin, adriamycin, and platinum in advanced thyroid cancer. *J Endocrinol Invest.* 1991;14:475-480.
67. Di Bartolomeo M, Bajetta E, Bochicchio AM, et al. A phase II trial of dacarbazine, fluorouracil and epirubicin in patients with neuroendocrine tumours. A study by the Italian Trials in Medical Oncology (I.T.M.O.) Group. *Ann Oncol.* 1995;6:77-79.
68. Wu LT, Averbuch SD, Ball DW, et al. Treatment of advanced medullary thyroid carcinoma with a combination of cyclophosphamide, vincristine, and dacarbazine. *Cancer.* 1994;73:432-436.
69. Orlandi F, Caraci P, Berruti A, et al. Chemotherapy with dacarbazine and 5-fluorouracil in advanced medullary thyroid cancer. *Ann Oncol.* 1994;5:763-765.
70. Modigliani E, Cohen R, Joannidis S, et al. Results of long-term continuous subcutaneous octreotide administration in 14 patients with medullary thyroid carcinoma. *Clin Endocrinol (Oxf).* 1992;36:183-186.
71. Frank-Raue K, Raue F, Ziegler R. Therapie des metastasierten medullären schilddrüsenkarzinoms mit dem somatostatinanalogon octreotide. *Med Klin.* 1995;90:63-66.
72. Smid WM, Dullaart RPF. Octreotide for medullary thyroid carcinoma associated diarrhoea. *Neth J Med.* 1992;40:240-243.
73. Bajetta E, Zilembo N, Di Bartolomeo M, et al. Treatment of metastatic carcinoids and other neuroendocrine tumors with recombinant interferon-alpha-2a. A study by the Italian Trials in Medical Oncology Group. *Cancer.* 1993;72:3099-3105.
74. Pyke CM, Hay ID, Goellner JR, et al. Prognostic significance of calcitonin immunoreactivity, amyloid staining, and flow cytometric DNA measurements in medullary thyroid carcinoma. *Surgery.* 1991;110:964-971.
75. Riddell DA, Lampe HB, Cramer H, Troster M. Medullary thyroid carcinoma: prognostic factors. *J Otolaryngol.* 1993;22:180-183.



## Thyroid Gland and Surgery of the Thyroglossal Duct: Exercise in Applied Embryology

Gregory M. Organ, M.D., Claude H. Organ, Jr., M.D.

Department of Surgery, University of California, Davis—East Bay Surgery Program, 1411 E. 31st Street, Oakland, California 94602, USA

**Abstract.** Thyroglossal duct cysts (TDCs), the most common congenital cervical abnormality, originates from the medial anlage of the thyroid gland and presents as a painless asymptomatic midline suprahyoid mass. It does not represent a diagnostic challenge. The tract may persist as a fibrous cord or leave nests of cells anywhere along its embryonic path, and it gives rise to the development of TDC. The Sistrunk operation described in 1920 consists of en bloc cystectomy and central hyoidectomy, with tract excision up to the foramen cecum. This procedure remains an effective treatment for TDC. Malignant degeneration of TDC is rare (0.7%).

Very early in fetal life the thyroid gland develops at the base of the tongue and before the cartilage of the hyoid bone has formed—W.E. Sistrunk [1]

Thyroglossal duct cysts (TDCs) are the most common congenital cervical abnormalities, three times more common than branchial cleft remnants. The cyst usually presents as a painless, asymptomatic midline swelling below the hyoid bone. (Fig. 1) TDCs may be observed at any age, but most are noted during childhood, usually by 5 years of age; the final third become apparent after age 30. TDCs are present at birth in approximately 25% of cases [2]. Unlike most thyroid disorders in which females predominate, the gender incidence is equal for TDCs. Cysts of the thyroglossal duct can be found anywhere in the midline from the submental region to the suprasternal notch but are most commonly located halfway between these extremes, near the hyoid bone. In their experience with 105 cases of TDC, Ward et al. noted that 80% were juxtaposed to the hyoid bone (25% located in the submental region), 2% lingual, and 7% in a suprasternal location. Only 1% of TDCs were lateral to the midline [3].

Thyroglossal duct cysts are round with a smooth surface and are well defined (Fig. 2). With swallowing or protrusion of the tongue, a TDC classically rises in the neck as a result of the cyst being anchored to the hyoid bone and muscles of the tongue. It is usually 1 to 2 cm in diameter, slightly mobile, and nontender unless there is superimposed infection. Typically oral bacteria are transmitted through the foramen cecum accompanied by erythema of the overlying skin. Thyroglossal duct sinuses are secondary to infection of the cyst as a result of spontaneous or surgical drainage and are associated with some degree of low grade inflammation of the surrounding skin. The cutaneous opening is

usually 1 to 3 mm in diameter and may intermittently express small droplets of thin mucoid fluid usually clear or yellowish [4, 5].

Histologically, a TDC is lined by pseudostratified ciliated columnar epithelium, squamous epithelium, or both. The supporting wall of the cyst consists of fibrous tissue and frequently contains heterotopic thyroid tissue (20%) and accumulations of other chronic inflammatory cells (Fig. 3). A TDC does not usually represent a diagnostic problem to the pathologist.

### Embryology of the Thyroid

The thyroid gland develops from the larger median anlage and the paired smaller lateral anlages. The median anlage, recognized by the end of the third week, forms the bulk of the thyroid gland. It presents on the ventral pharyngeal wall (the tuberculum impar) at the level of the second branchial arch, appearing as a single or paired diverticulum. Division of the gland into lateral lobes, if not present from the beginning, occurs so early that it is impossible to determine whether the human thyroid arises singly or as a paired organ. Although the median stalk usually has a lumen (the thyroglossal duct), it does not extend into the lateral lobes. The origin of the median anlage is marked by the foramen cecum. Early during the fifth week, the attenuated duct loses its lumen and shortly afterward breaks into fragments. The lateral thyroid anlage, which originates from the ventral portion of the fourth pharyngeal pouch, becomes attached to the posterior surface of the thyroid during the fifth week and contributes up to 30% to the thyroid weight. The causes of fusion of the median and lateral anlages are unknown [6]. Sugiyama speculated that the migration of the ultimobranchial body controls the growth of the median anlage, or that growth of the median anlage laterally and caudally inhibits future expansion of the ultimobranchial body. The lateral thyroid anlage provides the parafollicular C cells that produce calcitonin [7].

The thyroglossal duct is an epithelial tube that connects the gland and the foramen cecum. Early during the fifth week the duct loses its lumen and becomes attenuated into disconnected fragments. During the fifth through the seventh weeks of gestation the hyoid bone is formed by condensation of mesoderm with subsequent chondrification from the second and third branchial arches,

Correspondence to: C.H. Organ, Jr., M.D., e-mail: chorgan@ucdavis.edu

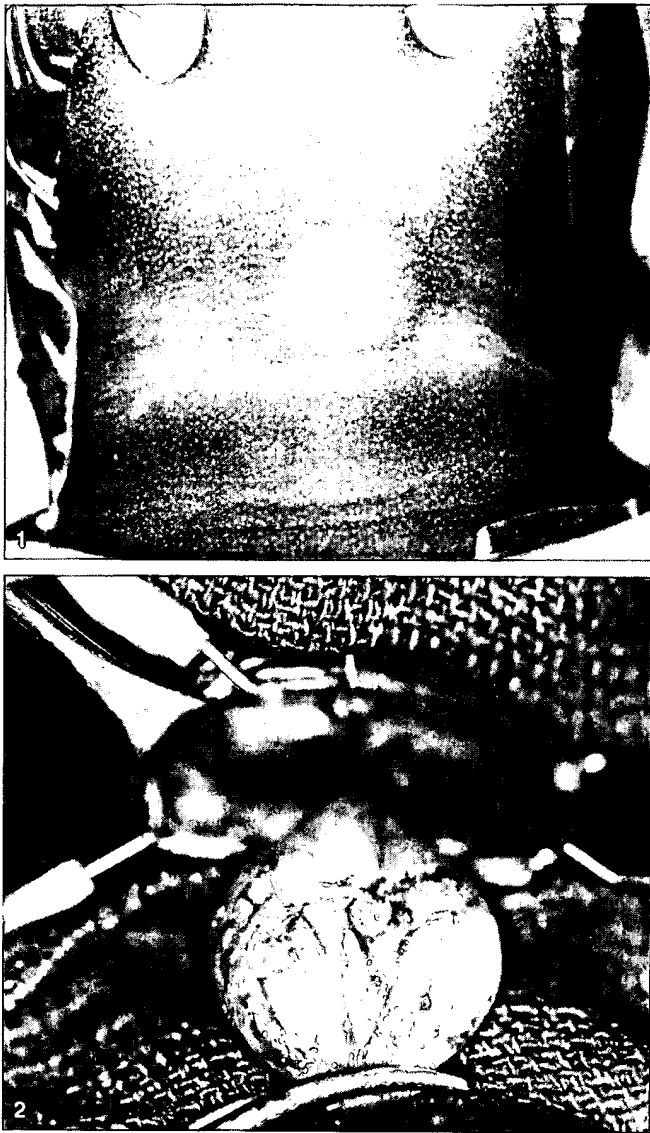


Fig. 1. Thyroglossal duct cyst in juxtaposition to the hyoid bone.

Fig. 2. Excision of thyroglossal duct cyst (magnified) with a well defined smooth surface accompanied by the suprahyoid tract.

which grow from behind forward, dividing the thyroglossal duct tract into suprahyoid and infrahyoid portions [2, 8, 9].

The attenuated thyroglossal duct tract usually atrophies and disappears by the end of the eighth week. This tract may persist as a fibrous cord or a minute epithelial tube. The thyroid gland may reach its normal position, leaving rests of cells anywhere along this embryonic path and give rise to postnatal development of cysts; or the entire organ may fail to complete the journey. If the entire gland fails to descend normally, it may lie close to its point of origin in the tongue (lingual thyroid) or at any level along the midline developmental pathway (sublingual, prelaryngeal, rarely suprasternal). TDCs never have a primary external opening because the embryologic course of the tract does not reach the surface of the neck. Horisawa et al. [10] have demonstrated by computed tomography (CT) (and have microscopically con-

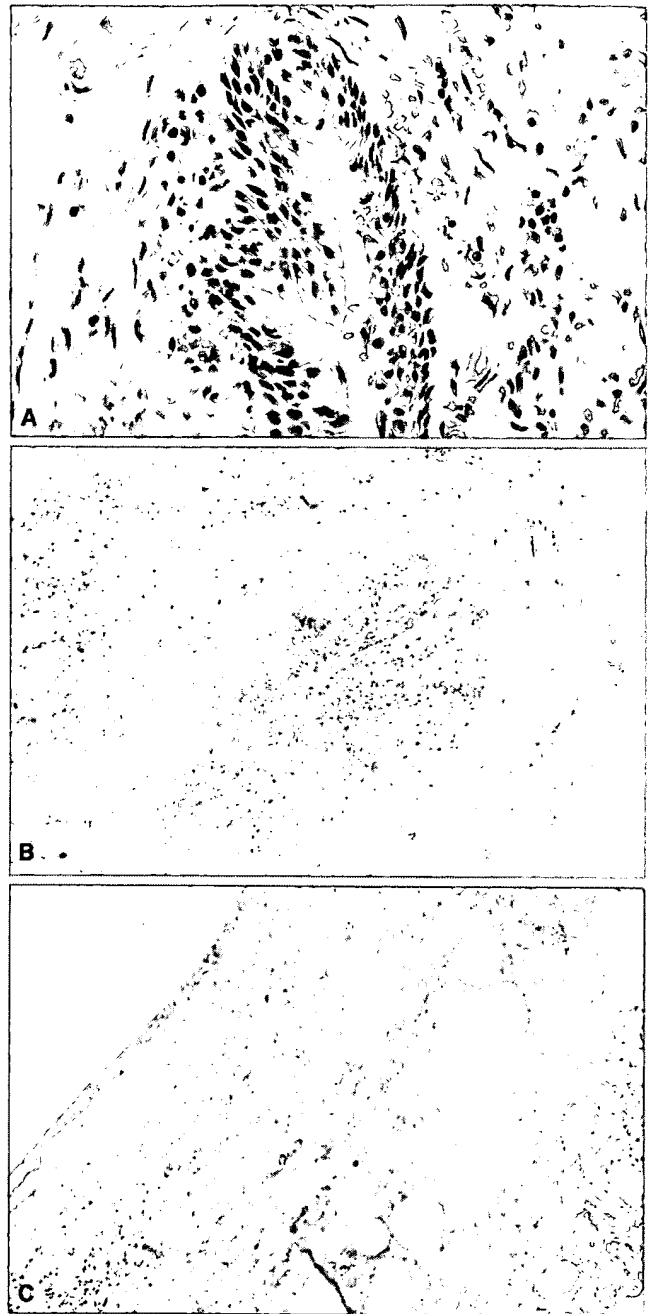


Fig. 3. A. High power view of a thyroglossal duct cyst exhibiting transitional epithelium and focal chronic inflammation. B. Thyroglossal duct cyst with transitional epithelium, chronic inflammation, and hemorrhage in the fibrous connective tissue wall. C. Thyroglossal duct wall exhibiting colloid-lined thyroid follicles.

firmed) penetration of the hyoid bone by the thyroglossal duct with ciliated epithelium.

Two pyramidal lobes may be present, suggesting that the primordium was paired. A pyramidal lobe is present in about half of individuals. Through lateral growth, two wings of tissue come to lie parallel and ventromedial to the elongating thymus glands, attached across the midline by the isthmus, to which the remains of the thyroglossal duct may attach as the pyramidal lobe [6].

The first follicles form from the epithelial plates at the beginning of the eighth week, and by the twelfth week the plates have been converted entirely into follicles. Subsequent follicle formation takes place by budding or division of primary follicles. Follicle formation is preceded by the appearance of an intracellular periodic acid-Schiff (PAS)-positive material. Bierring and Shepard have described intracellular canaliculi with microvilli, which open at the apex and are in contact with similar structures in adjacent cells [11]. These spaces become confluent and form the lumen of the developing follicles. Desmosomes connect the cells to keep the follicular contents from escaping. During the 11th and 12th weeks, all stages of follicle formation may be observed simultaneously. The greatest increase in follicle number takes place during the fourth month; colloid appears in the follicles during the 11th week. Evidence of thyroxine comes with the appearance of colloid [6].

LiVolsi divided the histologic differentiation of the human fetal thyroid into three stages: precolloid (7–13 weeks); colloid (13–14 weeks); and follicular (after 14 weeks) [12]. The final form of the thyroid gland is not constant. The isthmus is absent in about 10% of individuals. The left or right lobe may be small or completely absent; thyroidal hemiagenesis is rare [13].

### Surgical Management

The majority of operations for the cure of thyroglossal cysts are unsuccessful unless the epithelium-lined tract, running from the cyst to the foramen caecum, is completely removed—W.E. Sistrunk [1]

The original 1920 monograph by Walter Ellis Sistrunk (Fig. 4) from the Mayo Clinic described the surgical management of this congenital abnormality and remains the classic basic reference. Little has been added to Sistrunk's original report or to his follow-up manuscript, which contain no bibliographic references [14]. This monograph is "must" reading for surgeons who manage TDC. In his original report, 31 TDCs were encountered among 86,000 consecutive patients examined at the Mayo Clinic.

The "Sistrunk operation" consists of en bloc cystectomy and central hyoidectomy, with tract excision up to the foramen cecum. Sistrunk emphasized the need to make no attempt at isolating the suprahyoid portion of the duct. "At this point the tract usually passes through the hyoid bone, although it is sometimes found passing above or below it" [1]. The literature is saturated with reports that cystectomy alone without hyoidectomy and tract excision is the main cause of TDC recurrence.

The central core of tissue,  $\frac{1}{8}$  inch on all sides, is excised between the hyoid bone and foramen cecum. The index finger of the surgeon's hand should be inserted in the patient's mouth, placed on the foramen cecum to elevate the tongue and to guide excision of the suprahyoid tract. Sistrunk observed that the excision invariably proceeds at a 45-degree angle. The defect in the geniohyoid and mylohyoid muscles are reapproximated with absorbable suture, passed superficial to the cut surface of the muscle to avoid injury to the hypoglossal nerve. Although Sistrunk recommended apposition of the cut edges of the hyoid bone, it is more recently thought to be neither necessary nor wise to attempt to repair the cut ends of the hyoid, especially in a child [2]. Drainage of the wound is warranted in the presence of acute infection or operative disruption of the cysts.

Preoperative thyroid scintigraphy has been performed in pa-



Fig. 4. Walter E. Sistrunk, M.D. (1880–1933). (Mayo Clinic staff photograph, courtesy of The Mayo Clinic and Jon van Heerden, M.D.).

tients with presumed TDC to document the presence of a normal thyroid and to exclude the possibility of an ectopic thyroid mimicking a TDC. Preoperative sonography identifying a normal thyroid gland in patients with TDC confirms a source of thyroid hormone separate from the TDC and excludes ectopic thyroid tissue. Routine thyroid imaging is not necessary [15].

Approximately 1% of TDCs may undergo neoplastic change, 85% of which are papillary adenocarcinoma. Cases of squamous cell, anaplastic, and Hürthle cell carcinoma in TDCs have been reported [16]. Usually these malignancies are discovered following a Sistrunk procedure. Criteria for the diagnosis of primary papillary carcinoma arising in a TDC are (1) histologic identification of TDC demonstrating that the cyst or duct has an epithelial lining with normal thyroid follicles in the cyst wall; (2) there is normal thyroid tissue adjacent to the tumor; and (3) histopathologic examination of the thyroid gland reveals no signs of primary carcinoma [17]. Immunohistochemically, the cells covering the papillae stain strongly with anti-human macrophage/histiocyte antibodies (CD68 and MAC 387) but not with anti-keratin or anti-thyroglobulin antibodies [18].

Thyroglossal duct papillary carcinoma may represent a metastasis from a tumor of primary thyroid origin [19]. It is not known if (1) foci of carcinoma in the thyroid gland are metastases from primary carcinoma in the cyst; (2) two independent primary carcinomas exist; or (3) a primary thyroid cancer is the single source. Enlarged palpable cervical lymph nodes suggest malignancy before an operation. Differentiation of a TDC carcinoma from metastatic spread of a primary thyroid cancer is not always clear; hence management of TDC is controversial. Opinions on the need for radical surgery parallel those expressed regarding the treatment of differentiated thyroid carcinoma. Sistrunk's operation with a thorough follow-up is regarded as a minimum requirement.

The absence of Widstrom's diagnostic features does not exclude the possibility of thyroid involvement; hence total thyroidectomy is suggested by some investigators as the appropriate procedure for thyrogenic carcinomas. When carcinoma is found in a TDC, total thyroidectomy facilitates subsequent ablation of the thyroid remnant or distant metastatic lesions with radioactive iodine and permits better follow-up by thyroglobulin concentration measurements. Modified radical neck dissection is indicated only if there is metastatic lymph node involvement.

A 44-year experience reported from the Mayo Clinic revealed 12 patients with TDC carcinoma (6 males, 6 females) ranging in age from 17 to 60 years (mean 40 years). The frequency of carcinoma in TDCs was 0.7%. An upper midline neck mass was the initial sign in all patients. Eleven of twelve underwent a Sistrunk procedure, all of whom had papillary carcinoma; the thyroid was involved in three cases. Nine patients underwent subtotal or near-total thyroidectomy, three of whom received postoperative radioactive iodine. Their mean follow-up of 13 years revealed no documented local recurrences, distant metastatic involvement, or tumor-associated mortality. They concluded that TDC carcinoma is a rare malignant tumor, usually diagnosed postoperatively. Their recommended treatment: the Sistrunk procedure accompanied by a near-total or total thyroidectomy [20].

Hereditary TDCs are female-predominant and usually have an autosomal dominant pattern of inheritance. This gender bias may be explained by genetic imprinting. Although no racial differences have been noted with TDC, distinct variations in presentation based on nationality have been observed. The recurrence rate after a Sistrunk procedure is similar to that in nonhereditary cases [21].

Thyroglossal duct cysts may mimic laryngoceles, causing erosion of the thyroid cartilage lamina. These patients present with hoarseness and positional stridor. The anterior location deep to the strap muscles, the close proximity to the thyroid cartilage lamina, and preservation of the paraglottic fat plane help to differentiate it from other neck lesions [21].

When the TDC is lingual, respiratory stridor is the usual presenting symptom, rarely dysphagia. Improved respiratory symptoms can be expected when the infant is moved from the prone to the supine position. Other causes of congenital stridor should be excluded. The association of lingual TDC remnants in sudden infant death syndrome have been reported. The treatment for this lesion is the Sistrunk procedure or marsupialization. General anesthesia and intubation are preferable; however, tracheostomy may be necessary if intubation is difficult, or it may be performed prophylactically if postoperative edema at the base of the tongue is anticipated. Preferably the lesion is excised transorally, which is the simplest approach when the cyst is large. Marsupialization of

a lingual TDC (without excision) has resulted in no reported recurrences; there is an associated low morbidity rate and high success rate [22, 23]. Preoperative imaging studies may be helpful for delineating the extent of the cyst and confirming the presence of normal thyroid tissue in the neck. An adequate laryngoscopic examination is recommended to ensure the appropriate diagnosis. Because the cyst is located posteriorly, adequate exposure is critical. The low incidence of lingual TDC may be because the duct initially atrophies from the oral side when thyroid descent first begins.

Aspiration of the cyst alone or inadequate dissection is associated with rapid refilling of the cyst. The important aspects to this approach permit accurate diagnosis by laryngoscopy and imaging studies. Excellent exposure of the entire cyst during marsupialization and careful pre- and postoperative inspection for extension of the cyst into the neck are important. Repeat laryngoscopy is not recommended unless the child becomes symptomatic [23].

## Résumé

Les kystes du canal thyroïdienne (KCT), l'anomalie congénitale la plus fréquente rencontrée dans la région cervicale, prends son origine dans l'ébauche médiane de la glande thyroïde et se présente comme une masse médiane, sushyoïdienne, asymptomatique. Son diagnostic ne pose guère de problème. Le canal peut parfois persister sous la forme d'une corde fibreuse ou laisser tout au long de son trajet embryonnaire des foyers cellulaires qui peuvent donner naissance au développement d'un KCT. L'opération de Sistrunk, décrite en 1920, consiste en une kystectomie en bloc, une hyoïdectomie centrale avec excision du canal jusqu'au *foramen cecum* et constitue un traitement effectif du KCT. La dégénérescence maligne est rare (0.7%).

## Resumen

Los quistes del canal tirogloso, que constituyen la mayoría de las anomalías congénitas del cuello, ocurren en cualquier sitio del trayecto de emigración de la glándula tiroideas y se presentan clínicamente como una masa suprahiodea indolora y ubicada sobre la línea cervical media. No representan problema diagnóstico. El tracto puede persistir como un cordón fibrótico o puede dejar nidos de células a lo largo de su trayecto embrionario, los cuales dan origen a un quiste del canal tirogloso. La operación de Sistrunk, descrita en 1920, consiste en una resección en bloque del quiste, la resección de la porción central del hueso hioides y la resección del tracto hasta el foramen ciego de la lengua. Este procedimiento operatorio sigue siendo una modalidad efectiva en el tratamiento de los quistes tiroglosos. La degeneración maligna de un quiste tirogloso es poco común (0.7%).

## References

1. Sistrunk, W.E.: The surgical treatment of cysts of the thyroglossal tract. *Ann. Surg.* 71:121, 1920
2. Soper, R.T., Pringle, K.C.: Cysts and sinuses of the neck. In *Pediatric Surgery*, 4th ed., Welch, K., et al., editor, Chicago, Year Book, 1986, pp. 539-552
3. Ward, G.E., Hendrick, J.W., Chambers, R.G.: Thyroglossal duct abnormalities. *Surg. Gynecol. Obstet.* 89:729, 1949
4. Fallat, M.E.: Neck. In *Surgery of Infants and Children: Scientific Principles and Practice*, Oldham, K.T., Colombani, P.M., Foglia, R.P., editors, Philadelphia, Lippincott-Raven, 1997, pp. 835-855



5. Gross, R.E.: Thyroglossal cysts and sinuses. In *The Surgery of Infancy and Childhood*, Gross, R.E., editor, London, Saunders, 1953, pp. 936-946
6. Bloom and Fawcett: *A Textbook of Histology*, 12th ed., New York, Chapman & Hall, 1994, pp. 27-32
7. Sugiyama, S.: The embryology of the human thyroid gland including the ultimobranchial body and others related. *Ergeb. Anat. Entwicklungsgesch.* 44(H2):6, 1971
8. Gray, S.W., Skandalakis, J.E., Atkin, J.T.: Embryological considerations of thyroid surgery: developmental anatomy of the thyroid, parathyroids and the recurrent laryngeal nerve. *Am. Surg.* 42:621, 1976
9. Moore, K.L.: *The Developing Human*. Philadelphia, Saunders, 1977, pp. 69-70
10. Horisawa, M., Sasaki, J., Niinomi, N., Yamamoto, T., Ito, T.: Thyroglossal duct remnant penetrating the hyoid bone: a case report. *J. Pediatr. Surg.* 33:725, 1988
11. Bierring, F., Shepard, T.H.: The thyroid. In *Organogenesis*, DeHaan, R.L., Ursprung, H., editors, New York, Holt Rinehart & Winston, 1965, p. 493
12. LiVolsi, V.A.: *Surgical Pathology of the Thyroid*, Philadelphia, Saunders, 1990, pp. 3-6
13. Tsang, S.K., Maher, J.: Thyroid hemiagenesis accompanying a thyroglossal duct cyst: a case report. *Clin. Nucl. Med.* 23:229, 1988
14. Sistrunk, W.E.: Technique of removal of cysts and sinuses of the thyroglossal duct. *Surg. Gynecol. Obstet.* 46:109, 1928
15. Lim-Dunham, J.E., Feinstein, K.A., Yousefzadeh, D.K., Ben-Ami T.: Sonographic demonstration of a normal thyroid gland excludes ectopic thyroid in patients with thyroglossal duct cyst. *Am. J. Radiol.* 164:1489, 1995
16. Cote, D., Sturgin, E., Peterson, T., Miller, R.: Thyroglossal duct cyst carcinoma: an unusual case of Hürthle cell carcinoma. *Otol. Head Neck Surg.* 113:153, 1995
17. Widström, A., Magnusson, P., Halberg, O., Hellqvist, H., Riiber, H.: Adenocarcinoma originating in the thyroglossal duct. *Ann. Otol. Rhinol. Laryngol.* 85:286, 1976
18. Zidar, N., Gale, N.: Unusual inflamed thyroglossal cyst. *J. Laryngol. Otol.* 109:899, 1995
19. Kojima, Y., Sakata, H., Nakamura, Y., Furuya, N.: Papillary carcinoma of the thyroid originating in a thyroglossal cyst: report of a case and diagnostic criteria. *J. Otorhinolaryngol.* 58:238, 1996
20. Heshmati, H.M., Fatourehchi, V., van Heerden, J.A., Hay, I.D., Goellner, J.R.: Thyroglossal duct carcinoma: report of 12 cases. *Mayo Clin. Proc.* 72:315, 1997
21. Greinwald, J.H., Jr., Leichtman, L.G., Simko, E.J.: Hereditary thyroglossal duct cysts. *Arch. Otolaryngol. Head Neck Surg.* 122:1094, 1996
22. Urao, M., Teitelbaum, D.H., Miyano, T.: Lingual thyroglossal duct cyst: a unique surgical approach. *J. Pediatr. Surg.* 31:1574, 1996
23. Kuint, J., Horowitz, Z., Kugel, C., Toper, L., Birenbaum, E., Linder, N.: Laryngeal obstruction caused by lingual thyroglossal duct cyst presenting at birth. *Am. J. Perinatol.* 14:353, 1997



## Management of undifferentiated thyroid cancer

Kenneth B. Ain\* MD

Associate Professor of Medicine

Thyroid Nodule and Oncology Clinical Service, Division of Endocrinology and Molecular Medicine,

Department of Internal Medicine, University of Kentucky Medical Centre, Lexington, KY, 40536-0298, USA

Director

Thyroid Clinic and Thyroid Cancer Research Laboratory, Veterans Affairs Medical Centre, Lexington, KY, USA

Management of thyroid carcinoma relies upon the tumour cells maintaining the differentiated functions that are typical of normal thyroid follicular cells, such as: dependence upon thyrotropin for growth, production of thyroglobulin and effective transport of iodine. Likewise, differentiated thyroid carcinomas often exhibit an auspicious clinical behaviour with a slow rate of growth and low potential for invasion and distant metastasis. These features permit therapy of disseminated tumour, effective follow-up surveillance and the assumption of a good prognosis. As each of these features are lost, the opportunities for both disease status assessment and therapeutic intervention diminish accordingly. A major obstacle is our failure to define effective systemic treatments to replace radioiodine therapy, whose loss is consonant with the loss of iodine transport and retention. The extreme of undifferentiated clinical behaviour is epitomized by anaplastic thyroid carcinoma, a rare, terminally dedifferentiated malignancy that is rapidly and invariably fatal. It is important to be attuned to clinical clues suggesting the presence of dedifferentiated tumour and related prognostic signs. This allows the application of currently limited therapeutic options and defines the need for research to develop new systemic treatments.

**Key words:** thyroid neoplasms; cell differentiation; chemotherapy, adjuvant; radiotherapy, adjuvant; investigative techniques; anaplastic carcinoma.

### FEATURES OF DIFFERENTIATION AND THE CONSEQUENCES OF THEIR LOSS IN THYROID CANCER

Thyroid epithelial carcinomas are generally classified into papillary or follicular histological types, including their respective variants. In this context, differentiation refers to the maintenance of cellular functions that are particular to benign thyroid follicular cells, namely a slow growth rate and a low propensity to metastasize. It is also applied as a descriptive term to histological features that are typically associated with

\*Address for correspondence: Division of Endocrinology and Molecular Medicine, Room MN 520, Department of Internal Medicine, University of Kentucky, 800 Rose Street, Lexington, KY 40536-0298, USA.

differentiated cellular functions. Rather than serving as a strictly nosological tool, the degree of differentiation determines the likelihood of beneficial response to clearly limited therapeutic options that take advantage of thyroid-specific processes.

The genetic and epigenetic changes associated with dedifferentiation are under active investigation. Although our understanding of these changes is still very preliminary, it appears likely that some of them, particularly epigenetic changes, may be reversible. Also, the incremental nature of these changes is of immediate clinical relevance. For example, a gradual decline in iodide transport in metastases may still permit effective tumouricidal results with  $^{131}\text{I}$  therapy, provided that maximal doses (up to marrow tolerance) are employed with optimal patient preparation.<sup>1</sup> However, the recognition of the heterogeneous levels of expression of the iodide transporter (sodium/iodide symporter; NIS), amongst different cells within the same tumour, may set the stage for low doses of  $^{131}\text{I}$  therapy to provide a selection pressure for the loss of NIS expression and dedifferentiation in progeny cells. Thus, understanding these effects has value, both for treating dedifferentiated tumours and for preventing the evolutionary transformation of differentiated tumours to a non-functional phenotype.

An important point is that dedifferentiation is often disconjugate, with the loss of some functions and the retention of others. Discerning the status of different tumour functionalities is a critical part of any therapeutic strategy. This could enhance diagnostic sensitivity, the selection of appropriate treatments and may permit palliative measures, even in the absence of curative opportunities.

### Functional features

Malignant thyroid follicular cells typically express cell membrane receptors to thyrotropin (thyroid stimulating hormone; TSH) with an active transduction machinery that is capable of eliciting a cascade of a variety of second messengers. These stimulate progression through the cell cycle with enhancement of growth, elaboration of thyroglobulin and both production and membrane-targeting of NIS. This provides the rationale for both suppressing endogenous TSH with levothyroxine to reduce tumour growth and for transient hypothyroid-induced stimulation of TSH to enhance  $^{131}\text{I}$  delivery to malignant cells. Suppression of endogenous TSH is associated with improved patient outcomes<sup>2</sup> and it is possible that thyroid cancer cells may be less susceptible to TSH-response desensitization than normal thyroid follicular cells.<sup>3</sup> However, thyroid malignancies with histological evidence of dedifferentiation and a poor prognosis have diminished TSH receptors<sup>4</sup>, and show TSH-independent proliferation.

The ability to treat metastatic disease with radioactive iodine is unique to thyroid cancers with sufficient expression of NIS.<sup>5</sup> Although several other tissues express this gene at low levels, it appears to be important for the appropriate membrane placement of NIS protein for optimal activity of this pump.<sup>6</sup> These processes alone are insufficient to retain radioiodine within the tumour cells long enough to deliver tumouricidal radiation doses, as shown by NIS gene transfection studies.<sup>7</sup> Retention requires organification of iodine, mediated partly by a differentiation-dependent thyroid-specific product, thyroid peroxidase (TPO). Thyroidal TPO expression is diminished by malignant transformation<sup>8</sup> and may account for a rapid loss of accumulated radioiodide, which is probably responsible for some treatment failures.<sup>9</sup> Sometimes retention of radioiodine inside tumour cells may be enhanced by the administration of lithium carbonate, as shown in clinical trials<sup>10</sup>, although this agent does not increase iodine uptake.

Thyroglobulin is the major component of thyroidal colloid and is unique to thyroid follicular cells. There is no evidence of illegitimate transcription of its gene in any other human tissue. Like other thyroid cell proteins, its expression may be diminished in dedifferentiated malignancies<sup>11</sup>; however, it typically continues to be expressed despite the loss of NIS and/or TPO. This often permits it to serve as a reliable marker for persistent or recurrent tumour, even in the absence of radiologically-detectable gross disease.<sup>12</sup> The presence of anti-thyroglobulin autoantibodies in patients may diminish the diagnostic reliability of the serum thyroglobulin assay; however, new tests, assessing peripheral blood for the presence of circulating thyroid cancer cells by converting their thyroglobulin messenger RNA into complementary DNA that is amplified by the polymerase chain reaction and quantitatively assessed, have suggested for higher sensitivities for the diagnosis of persistent thyroid cancer.<sup>13</sup>

Differentiation embodies the behaviours of slow tumour growth, limited invasion and rare metastases. The maximal dedifferentiated behaviour is exemplified by anaplastic thyroid carcinoma (ATC) with clinical doubling times as short as 2 days and invariable metastatic dissemination. The full spectrum between these behaviours can be seen in dedifferentiated tumours, with disease progression ranging over time periods as short as months to as long as decades. These differences are likely to be intrinsic to the degree of histological differentiation<sup>14</sup> and may be consequent to the expression of a variety of distinct gene products, such as E-cadherin.<sup>15</sup> E-cadherin expression is also affected by epigenetic factors related to tumour differentiation status<sup>16</sup> and may be reversible.<sup>17</sup> Further identification of additional mechanisms, particularly those altering progression through the cell cycle, should provide new rationales for innovative therapeutics.

#### Histological features

Histological features that imply the loss of differentiation with a resulting poor prognosis include: extrathyroidal invasion, DNA aneuploidy, the presence of a solid, trabecular, or scirrhous pattern, distant metastases and bulky or invasive nodal metastases.<sup>18-21</sup> Tumours with these features may lose differentiated functions as an initial or later development, although some tumours retain these functions.<sup>22,23</sup> Pathologists have denoted histological subtypes that are suggestive of aggressive tumour behaviour and the loss of differentiated functions. These include tall cell variant papillary cancers, which frequently lose iodide uptake<sup>24</sup> via potentially reversible mechanisms.<sup>25</sup> Similar associations have been suggested for columnar cell variants<sup>26</sup>, oxyphilic (Hürthle-cell) variants<sup>27,28</sup> and insular carcinomas.<sup>29</sup> Careful analysis of primary tumour histology may presage tumour dedifferentiation and focus the clinical approach. However, a retrospective evaluation of patients with distantly metastatic, dedifferentiated tumours failed to discern histological features that were distinct from many well-differentiated, therapeutically responsive cancers.<sup>22</sup>

#### Clinical consequences of dedifferentiation

The loss of thyroid-specific functions impedes both diagnostic and therapeutic efforts. These efforts rely upon the expression of NIS and thyroglobulin, permitting the use of radioiodide, as well as the measurement of circulating thyroglobulin. In most cases, tumours secrete thyroglobulin despite failing to concentrate iodide, providing important clues that denote the presence of dedifferentiated carcinoma.<sup>30</sup> This should prompt the use of alternative imaging strategies to find tumour sites, such as

computerized axial tomographic scans, magnetic resonance imaging, or alternative nuclear medicine techniques. Further dedifferentiation may diminish thyroglobulin secretion, potentially lulling the clinician into complacency in the absence of obvious gross tumour. Astute diagnostic management incorporates multiple complementary imaging methodologies so that tumour progression is detected as early as possible and, hopefully, as localized and resectable disease. This is critical because there are no effective systemic therapies aside from radioiodine.

Differentiation status inversely correlates with the tumour growth rate. The extreme example is anaplastic thyroid carcinoma, which has extraordinarily brief doubling times. Although growth of differentiated cancers is partly TSH-dependent, dedifferentiation may result in diminished expression of TSH receptors<sup>4</sup> as well as diminished signal transduction after receptor activation.<sup>31</sup> In addition, other uncharacterized growth factors may exert greater influence on disease progression<sup>32</sup>, but it is not easy to determine which patients no longer benefit from suppression of their TSH. For this reason, suppressive use of levothyroxine is still advised when differentiated features are lost.

### Anaplastic thyroid carcinoma

Anaplastic thyroid carcinoma (ATC) is the ultimate dedifferentiation of thyroid carcinomas, since the consequence of transformation to this tumour type is an inexorable fatal outcome.<sup>33</sup> Primary prevention of ATC involves successful clinical management of precursor differentiated tumours and is always more successful than treatment of consequent ATC. ATC cells do not have effective iodide retention sufficient to respond to radioiodine therapy although they express NIS mRNA.<sup>34</sup> Assertive control of local disease with surgery and external beam radiotherapy is usually helpful, but ultimately fails due to distant metastases and the absence of effective systemic therapies. Recent preclinical experiments have suggested activity of paclitaxel against ATC<sup>35</sup> and this has been verified in a clinical trial.<sup>36</sup> This agent merely delays mortality in responsive patients and is only a first step in an arduous journey of future experimental therapeutics.

## CLINICAL APPROACHES TO UNDIFFERENTIATED TUMOURS

The fundamental failure of clinical management of undifferentiated thyroid cancer is the absence of any demonstrably effective systemic therapy. Treatment strategies must make optimal use of inadequate modalities in rare curative efforts and more common palliative attempts. The first step is to make certain that iodide uptake is truly lost, rather than reflecting inadequate preparation or stable iodide contamination. Many clinicians do not appreciate the fact that interference with radioiodine uptake in metastases from stable iodine in radiographic contrast media may persist for nearly a year.<sup>37</sup> In some cases, patients with 'dedifferentiated' tumours demonstrate excellent radioiodide uptake after providing sufficient time for clearance of stable iodide and careful preparation for treatment with a strict low-iodine diet.<sup>38</sup>

The next step is to identify those patients with localized disease who may be treated with surgical resection and external radiotherapy (XRT). There may be a narrow 'window of opportunity' for locoregional disease to respond to such management before distant dissemination. Such patients should be discriminated from those with

uni  
be

As

Th  
un  
to  
tu  
nt  
cy  
ur  
io  
<  
d  
d

ti  
F  
d  
/

t

ng, or alternative  
ish thyroglobulin  
bsence of obvious  
e complementary  
ly as possible and,  
use there are no

growth rate. The  
raordinarily brief  
y TSH-dependent,  
eptors<sup>4</sup> as well as  
n, other uncharac-  
ression<sup>32</sup>, but it is  
ssion of their TSH.  
hen differentiated

iation of thyroid  
umour type is an  
successful clinical  
ore successful than  
e iodide retention  
ess NIS mRNA.<sup>34</sup>  
am radiotherapy is  
nd the absence of  
uggested activity of  
This agent merely  
arduous journey of

## TUMOURS

nd thyroid cancer is  
nent strategies must  
and more common  
uptake is truly lost,  
ontamination. Many  
iodine uptake in  
persist for nearly a  
monstrate excellent  
of stable iodide and

who may be treated  
e may be a narrow  
o such management  
ed from those with

unresectable local or distant disease, who require expert clinical guidance and should be considered for creative approaches and innovative experimental studies.

### Assessment of tumour function for clinical intervention

Thyroid cancers may dedifferentiate before or after initial diagnosis. Apart from clearly undifferentiated anaplastic cancers, this is usually recognized after surgical thyroidectomy and radioiodine ablation and scanning. In some cases, gross local or distant tumour is evident from radiographic studies and does not concentrate radioiodine on nuclear scans. It may be necessary to verify these metastases by biopsy (and immunocytochemistry for thyroglobulin) to rule out coincident unrelated cancers. As long as urinary iodine studies suggest that this is not a false-negative finding due to stable iodine contamination ( $< 100 \mu\text{g}$  iodide in a 24-h urine collection is minimally adequate,  $< 50 \mu\text{g}$  is preferred) the negative results of the diagnostic scan should be sufficient to define the nature of the cancer as unresponsive to radioiodine, channelling further clinical efforts differently.

In other patients, with elevated thyroglobulin values and no radiological evidence of tumour sites, the administration of a large  $^{131}\text{I}$  treatment dose ( $> 150 \text{ mCi}$  (5.5 GBq)) permits a whole body scan 2–6 days after the  $^{131}\text{I}$  time of administration. This scan can demonstrate tumour sites for permitting surgical treatment of localized tumour. Alternatively, this treatment dose may result in effective lowering of serum thyroglobulin, suggesting therapeutic benefit.<sup>12,39,40</sup>

### Approach to the patient with elevated thyroglobulin and negative $^{131}\text{I}$ scans

Figure 1 depicts an approach for resolving the dilemma of persistent disease (as shown by elevated circulating thyroglobulin), with no non-physiological sites of uptake on  $^{131}\text{I}$  whole body scanning and no other indication of metastatic sites. The first task is to verify that scan results are reliable by ruling out excessive stable iodine with a 24-h urine sample for iodine following 1 week of a low iodine diet. After such verification, using hypothyroidism for preparation ( $\text{TSH} > 30 \text{ mIU/m}^4$ ) and a low iodine diet, the patient is given an  $^{131}\text{I}$  dose exceeding  $150 \text{ mCi}$  (5.5 GBq). Euthyroid scan preparation with recombinant human TSH is not advised because it is possible that such a preparation provides an insufficient length of time of TSH stimulation of potentially dedifferentiated tumour and the resulting body scan is significantly less sensitive. The post-therapy whole body nuclear scan may denote the sites of metastatic disease. If so, these sites may be resectable (such as in the neck or as isolated lung metastases). If unresectable, then they should be approached as described in Figure 2.

In the case of a negative post-therapy  $^{131}\text{I}$  whole body scan, thyroglobulin levels (stimulated by hypothyroidism or recombinant human TSH) should be assessed several months later to determine whether a therapeutic benefit was gained despite the absence of tumour localization on the scan. If thyroglobulin is significantly decreased, further  $^{131}\text{I}$  therapy should be considered. In any case, a number of tumour localization procedures should be employed. We find that extensive ultrasound evaluation of the neck, coupled with fine needle biopsies of all suspicious sites, is the most valuable initial technique for finding tumour in this situation. Computerized axial tomography of the lung or magnetic resonance imaging of the mediastinum, abdomen and pelvis are also useful. Recent studies have described the value of alternative nuclear scanning agents and positron emission tomography with 18-fluorodeoxyglucose to find tumour

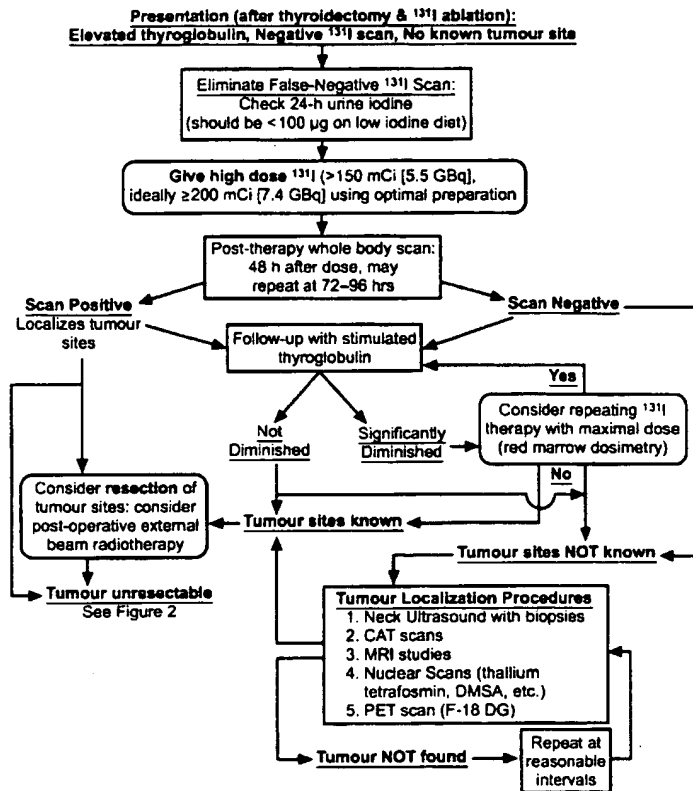


Figure 1. Clinical flow chart for unknown tumour site. CAT, computerized axial tomography; MRI, magnetic resonance imaging; DMSA, dimercaptosuccinic acid; PET, positron emission tomography; F-18 DG, (18-F) fluorodeoxyglucose.

sites.<sup>42</sup> These studies should be repeated at reasonable intervals, particularly if thyroglobulin values are increasing, to try to determine disease sites.

#### Approach to the patient with gross dedifferentiated tumour

Figure 2 describes the management of macroscopic, dedifferentiated thyroid cancer. As in any situation in which metastatic thyroid cancer fails to concentrate radioiodine on scanning, it is important to rule out false-negative results from stable iodine contamination (similar to Figure 1). The next issue is to confirm the tumour as thyroid cancer. It is not rare for cancer patients to harbour more than one primary malignancy and thyroid cancer patients appear to be at risk for other malignancies such as breast cancer.<sup>43</sup> If thyroglobulin values are severely elevated, this is supportive of the hypothesis that the metastatic tumour is thyroidal; however, moderate or low levels of thyroglobulin leave room for doubt. Such doubt should be eliminated by biopsy and immunohistochemistry for thyroglobulin.

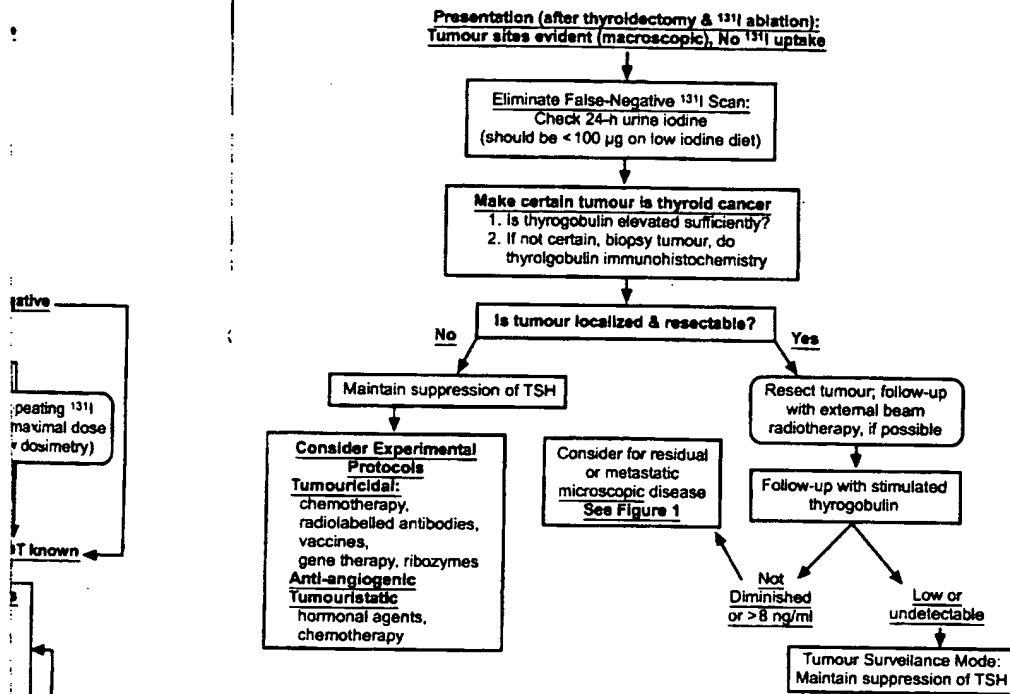


Figure 2. Clinical flow chart for macroscopic dedifferentiated tumour. TSH, thyroid stimulating hormone, or thyrotropin.

After appropriate confirmation, tumour sites should be assessed for resectability and operable gross tumour should be removed. Post-surgical external beam radiotherapy often provides additional anti-neoplastic control if able to be safely directed to localized regions.<sup>44,45</sup> Stimulated thyroglobulin levels (by hypothyroidism or recombinant human TSH injections), as well as continued radiographical studies, provide evidence of treatment effectiveness. If thyroglobulin levels are elevated, despite resolution of gross tumour, the patient should be further evaluated (as in Figure 1) since this may represent concomitant micrometastatic disease or tumour in occult sites that may benefit from high dose radioiodine treatment. If stimulated thyroglobulin levels become extremely low or undetectable, the patient should be maintained on TSH-suppressive levothyroxine dosages and periodically evaluated in a tumour surveillance mode.

Patients with macroscopic, unresectable thyroid carcinomas that do not concentrate radioiodine, constitute the major therapeutic dilemma. Since there are no established effective treatment modalities, these patients are very appropriate subjects for prospective clinical trials of innovative therapies. The rate of tumour progression may be slow enough for survival to extend over several years and some patients have avoided significant morbidity for more than a decade. In this context, aggressive unproven chemotherapeutics with severe side effects should not be used unless specific new preclinical data justifies a formal research trial. Patients may coexist



reasonably with their cancer, provided that metastases do not progress in critical areas, such as: the central nervous system, tracheal lumen, mainstem bronchus<sup>46</sup>, or other vital places.<sup>47</sup> Such dangerous metastases should be surgically resected or considered for stereotactic radiotherapy ('gamma knife'). However, rapidly progressive disease frequently results in desperate treatments with a variety of anti-neoplastics. It may be difficult for both the physician and the patient to resist the intense psychological pressures to 'do something' when there is nothing known to do. Palliative procedures, such as arterial embolization of metastases<sup>48</sup>, laser surgery of intraluminal tracheal tumours, and pleurodesis of malignant pleural effusions, may be very effective for lengthening and enhancing the quality of life.

#### **The rationale for resection of macrometastatic disease**

Patient safety and survival dictates the resection of macrometastatic disease in critical sites, such as the brain, spinal cord, or bronchus; however, resection of even non-critical metastases can be justified for biological reasons. The local environment within a tumour metastasis contributes to therapeutic failure. Intratumoural vasculature is tortuous with flawed endothelial linings and arterio-venous shunting resulting in sluggish and fluctuating blood flow.<sup>49</sup> This contributes to ischaemia and hypoxia in tumour regions between blood vessels, enhancing radiation resistance and resistance to chemotherapy agents. Hypoxia induces p53-mediated apoptosis<sup>50</sup>, providing a constant selection pressure for p53 mutations and resulting in further dedifferentiation and aggressive clinical behaviour. Ischaemic tumour cells are likely to become unresponsive to radioiodine therapy because of diminished access to circulating radioiodine, increased radioresistance and probably decreased NIS expression. Additionally, mutational events are more likely when the tumour burden is greatest.

#### **Alternative systemic therapies (experimental and speculative)**

##### *Chemotherapy*

Systemic chemotherapies in patients with thyroid carcinoma have been uniformly ineffective despite numerous, usually anecdotal, trials.<sup>51</sup> The agents and regimens used have included combinations of: doxorubicin, cisplatin and bleomycin<sup>52</sup>; doxorubicin, cisplatin and vindesine<sup>53</sup>; and single agents, including, mitoxantrone<sup>54</sup>, aclarubicin<sup>55</sup> and doxorubicin.<sup>56</sup> Although paclitaxel has significant activity in ATC<sup>35,36</sup>, our experiences in non-ATC, dedifferentiated thyroid cancers have been less rewarding.

The ineffectiveness of anti-neoplastic agents in thyroid cancer suggests an active role for one or more mechanisms associated with chemotherapy resistance. Satake et al<sup>57</sup> investigated the expression of P-glycoprotein (a MDRI gene product), multidrug resistance-associated protein (MRP; a MRPI gene product), LRP (lung resistance protein; major vault) and mutations of DNA topoisomerase II- $\alpha$  (TOPII) in several examples of ATC cell lines and tumours. They found that all expressed MRP, most expressed LRP and less than half expressed MDRI. All expressed TOPII and there were no mutations in this gene. P-glycoprotein and MRP are membrane proteins that pump chemotherapy agents out of cells, resulting in resistance to those agents. MRP has a similar spectrum of drug transport activity to P-glycoprotein, except that it is less able to transport paclitaxel or colchicine.<sup>58</sup> LRP has similar transport properties in the membranes of intracellular vesicles, resulting in sequestration and exocytosis of chemotherapy agents, while mutations in TOPII may alter this potential drug target.<sup>59</sup>

ess in critical  
ronchus<sup>46</sup>, or  
resected or  
y progressive  
neoplastics. It  
tense psycho-  
do. Palliative  
if intraluminal  
very effective

ase in critical  
tion of even  
environment  
neuronal vascul-  
ating resulting  
and hypoxia in  
and resistance  
providing a con-  
differentiation  
y to become  
ulating radio-  
Additionally,

een uniformly  
regimens used  
doxorubicin,  
arubicin<sup>55</sup> and  
ur experiences

an active role  
Satake et al<sup>57</sup>  
ct), multidrug  
ing resistance  
pPIL) in several  
ed MRP, most  
pPIL and there  
proteins that  
e agents. MRP  
cept that it is  
properties in  
exocytosis of  
drug target.<sup>59</sup>

We found roughly similar results, with uniformly high expression of MRP but no expression of MDRI mRNA.<sup>60</sup> As well as serving as an explanation for the failure of systemic anti-neoplastic therapies, these results suggest that MRP and LRP may provide appropriate targets for inactivation, in order to restore a clinical response to standard chemotherapies. This is the rationale for active research efforts to find pharmacological inhibitors of these chemotherapy pumps.

Recent clinical trials have made use of new drugs capable of inhibiting the growth of blood vessels into tumours, making large tumour masses haemodynamically unstable and inhibiting tumour growth. Although active preclinical studies are underway in our laboratory with a variety of agents, published studies are rare.<sup>61</sup> It is likely that the best results will require anti-angiogenesis agents used in combination with cytotoxic chemotherapy.

#### *Restoration of radioiodine uptake*

Since the most effective systemic therapy for differentiated thyroid cancer has been radioiodine, it is rational to attempt to restore the components of iodide uptake and retention which make this possible in dedifferentiated tumours. One of the earliest compounds advocated for this purpose was 13-cis retinoic acid (13cRA). The recent review by Schmutzler and Köhrle<sup>62</sup> summarizes the current published experiences. Careful analysis of these cases arouses significant scepticism, particularly since *in vivo* studies have suggested that retinoids inhibit radioiodine uptake<sup>63</sup>, inhibit thyroid peroxidase expression<sup>64</sup>, inhibit thyroglobulin expression<sup>65</sup> and do not restore radioiodide uptake despite increased NIS mRNA.<sup>66</sup> In addition, most published patient reports did not have an absolute lack of iodide transport in tumours prior to starting 13cRA and diets were not controlled for stable iodine contamination. To assess this we completed a prospective clinical trial of 13cRA under tightly controlled circumstances which failed to demonstrate any restoration of uptake in such tumours (unpublished results). Although clinicians appear to be trying this agent in some patients with dedifferentiated tumours, there is no objective evidence presented that this approach successfully restores tumour radioiodine uptake.

We have explored the molecular biology of NIS expression and found evidence to implicate epigenetic changes as being responsible for the loss of NIS expression and function. These studies suggest that methylation of cytosine residues in CpG DNA sequences in the regulatory regions of the NIS promoter, or portions of the first NIS intron, may contribute to transcriptional repression resulting in the loss of iodide uptake. Human thyroid carcinoma cell lines, without expression of NIS mRNA nor measurable radioiodine uptake *in vitro*, were treated with 5-azacytidine (an inhibitor of DNA-methyltransferase) resulting in the restoration of NIS mRNA expression and radioiodine uptake.<sup>25</sup> This agent is now under active investigation, in a phase Ib clinical trial, to attempt the restoration of radioiodine uptake in tumours of patients with metastatic dedifferentiated thyroid cancer, thus enabling radioiodine therapy to be used. Additional agents, which enhance transcription by altering chromatin structure, are under development for similar experimental clinical applications, including regulators of polyamine synthesis<sup>67</sup> and histone deacetylase inhibitors.<sup>68</sup>

#### *Gene transfer therapy*

Gene transfer experiments, in which the NIS gene is transfected into dedifferentiated thyroid cancer cells<sup>7</sup> or non-thyroidal malignancies<sup>69-71</sup>, also attempt to restore or

create therapeutic responsiveness to radioiodine. Major obstacles to the clinical application of this approach include: problems inherent to the current viral vectors, the difficulty of specifically targeting metastatic tumour cells<sup>72</sup>, and the need to restore cellular components of iodide organification and retention in order to deliver sufficient tumouricidal radiation. One potential way of targeting specific tumour cells is to use a tumour-specific promoter to limit the expression of transfected NIS to tumour cells despite systemic gene therapy. The thyroglobulin promoter is a possible candidate for thyroid cancer cells, for targeted expression of NIS or alternative suicide genes<sup>73</sup>, while the prostate-specific antigen promoter provides targeted NIS expression in prostate cancer cells stimulated with androgens.<sup>74</sup>

The tumour suppressor gene, p53, is typically mutated or epigenetically repressed in dedifferentiated thyroid carcinomas.<sup>75</sup> Transfection of this gene into ATC cells decreases growth rates and restores responsiveness to chemotherapy drugs<sup>76</sup>, as well as enhancing radiosensitivity<sup>77</sup>, thyroid peroxidase expression<sup>78</sup>, responses to TSH<sup>79</sup> and immunoreactivity.<sup>80</sup> This gene is particularly attractive as a therapeutic target because it should not require tumour cell-specific targeting, since there is no known deleterious effect of wild-type p53 on normal cells, and new evidence suggests that p53 mutations may be compensated for by pharmacological agents.<sup>81</sup> Additional genetic therapies may make use of ribozymes, which are enzymes that cleave RNA molecules in the cell, preventing them from producing specific proteins. Synthetic hammerhead ribozymes can be produced, which precisely destroy the mutant RNA species responsible for the malignant behaviour of cancer cells.<sup>73</sup> Likewise, other ribozymes can be engineered to repair mutant p53 gene products.<sup>82</sup>

#### *Hormonal therapy*

Somatostatin is a hormone with a multitude of effects, including a potential for tumour growth inhibition. These effects are mediated through five distinct subtypes of somatostatin receptors (SSTR1–SSTR5). Early studies documented binding of radiolabelled somatostatin to thyroid cancer cells, as well as unusual and contradictory responses of human thyroid cancer cell lines to two different somatostatin analogs.<sup>83</sup> Later investigations denoted SSTR3 and SSSTR5 as the predominant somatostatin receptors expressed in thyroid cancers.<sup>84</sup> Clinical experience with somatostatin analogs has been largely limited to octreotide, with greatest affinity for SSTR2. Unfortunately, our clinical experience demonstrates that this analog increases or has no effect on the growth rate of dedifferentiated thyroid cancers, a finding further substantiated by Zlock et al.<sup>85</sup> Our preclinical studies of human thyroid carcinoma cell lines, grown as xenografts in nude mice, have failed to reveal significant growth inhibitory activity of a wide variety of somatostatin analogs, including some with a specific affinity for SSTR5. Our experience with radiolabelled octreotide as a nuclear medicine imaging agent for thyroid carcinoma has not been fruitful. It is likely that positive reports of its utility have been consequent to binding of the octreotide to SSTR2-expressing vascular endothelial cells, rather than directly to tumour cells.

#### **SUMMARY**

The absence of effective systemic therapies for metastatic dedifferentiated thyroid carcinomas is the major impediment to their successful clinical management. Current clinical strategies employ aggressive control of locoregional disease with surgery and

es to the clinical  
rent viral vectors,  
the need to restore  
order to deliver  
specific tumour cells  
transfected NIS to  
motor is a possible  
alternative suicide  
targeted NIS expres-

netically repressed  
ne into ATC cells  
py drugs<sup>76</sup>, as well  
responses to TSH<sup>79</sup>  
therapeutic target  
there is no known  
e suggests that p53  
Additional genetic  
ve RNA molecules  
hetic hammerhead  
tant RNA species  
, other ribozymes

potential for tumour  
distinct subtypes of  
binding of radio-  
and contradictory  
statostatin analogs.<sup>83</sup>  
inant somatostatin  
with somatostatin  
affinity for SSTR2.  
og increases or has  
, a finding further  
roid carcinoma cell  
significant growth  
uding some with a  
eotide as a nuclear  
ful. It is likely that  
the octreotide to  
tumour cells.

erentiated thyroid  
agement. Current  
e with surgery and

### Practice points

- radioiodine scanning studies are insufficient for documenting the absence of persistent or recurrent thyroid carcinoma in dedifferentiated cancers. They must be supplemented with thyroglobulin levels, radiological studies and additional nuclear studies using different imaging agents
- the low iodine diet is a critical component of preparation for radioiodine scanning and therapy. Likewise, a single iodinated contrast media injection can interfere with such scanning and therapy for much of a year. A 24-h urine iodine measurement while on a low iodine diet for 1 week documents the clearance of this source of stable iodine
- careful attention to the details of the primary tumour pathology may predict later tumour dedifferentiation
- if they are localized, dedifferentiated tumours should be resected as fully as possible and followed up with external beam radiotherapy
- it is important to maintain sufficient levothyroxine therapy to keep TSH values suppressed to values of under 0.1 mIU
- even in the case of widespread distant metastases, patients may have prolonged survival, provided that metastases in critical sites (such as the brain, intraluminal bronchus, spinal cord, etc) are aggressively treated
- resection of macrometastatic disease provides theoretical advantages of delaying further dedifferentiation
- there are currently no verified effective systemic therapies for metastatic thyroid cancers that do not concentrate radioiodine
- management of anaplastic thyroid carcinoma requires local disease control with surgical resection and external beam radiotherapy, followed by monitoring for distant metastases and implementation of systemic chemotherapy with paclitaxel. This may delay, but is not likely to prevent, disease-specific mortality

### Research agenda

- new systemic tumouricidal agents with activity against thyroid carcinomas are needed
- investigation of the causes of chemotherapy resistance may provide strategies for restoring the clinical activity of currently available chemotherapy agents
- studies of the mechanisms of epigenetic regulation of iodide transport in dedifferentiated thyroid cancers may provide methods of restoring clinical responsiveness to radioiodine
- dedifferentiated thyroid cancer is a particularly good model system for the development and implementation of different approaches to gene transfer therapy

external radiotherapy. Debulking metastatic tumours and ablation of disease in dangerous sites, such as the brain, may prolong survival. It is possible that reversible epigenetic changes may be responsible for the loss of differentiated functions. Ongoing preclinical and clinical trials may define the role of pharmacological agents in reversing these inactivating epigenetic changes. Additional investigations should focus on developing effective tumouricidal anti-neoplastic therapies. This may also involve using

anti-angiogenesis agents and possibly gene therapies. Management of this cancer requires careful application of a host of surgical and radiotherapy techniques, which are all often insufficient to resolve disease, but may enhance the quality of life and prolong the length of survival.

### Acknowledgement

The author is the recipient of research support from the National Cancer Institute of the National Institutes of Health and the United States Department of Veterans Affairs.

### REFERENCES

- \* 1. Benua RS & Leeper RD. A method and rationale for treating metastatic thyroid carcinoma with the largest safe dose of  $^{131}\text{I}$ . In Medeiros-Neto G & Gaian E (eds) *Frontiers in Thyroidology*, pp 1317–1321. New York: Plenum Medical Book Co, 1986.
2. Pujol P, Daurès J-P, Nsakala N et al. Degree of thyrotropin suppression as a prognostic determinant in differentiated thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 1996; 81: 4318–4323.
3. Al-Sobhi SS, Soh EY, Wong MG et al. Desensitization in normal and neoplastic human thyroid cell lines. *World Journal of Surgery* 1998; 22: 552–557.
4. Tanaka K, Inoue H, Miki H et al. Relationship between prognostic score and thyrotropin receptor (TSH-R) in papillary thyroid carcinoma: immunohistochemical detection of TSH-R. *British Journal of Cancer* 1997; 76: 594–599.
5. Jhiang SM, Cho J-Y, Ryu K-Y et al. An immunohistochemical study of  $\text{Na}^+/\text{I}^-$  symporter in human thyroid tissues and salivary gland tissues. *Endocrinology* 1998; 139: 4416–4419.
6. Kaminsky SM, Levy O, Salvador C, Dai G & Carrasco N.  $\text{Na}^+/\text{I}^-$  symport activity is present in membrane vesicles from thyrotropin-deprived non- $\text{I}^-$ -transporting cultured thyroid cells. *Proceedings of the National Academy of Sciences of the USA* 1994; 91: 3789–3793.
7. Shimura H, Haraguchi K, Miyazaki A, Endo T & Onaya T. Iodide uptake and experimental  $^{131}\text{I}$  therapy in transplanted undifferentiated thyroid cancer cells expressing the  $\text{Na}^+/\text{I}^-$  symporter gene. *Endocrinology* 1997; 138: 4493–4496.
8. Takamatsu J, Hosoya T, Tsuji M et al. Peroxidase and coupling activities of thyroid peroxidase in benign and malignant thyroid tumor tissues. *Thyroid* 1992; 2: 193–196.
- \* 9. Filetti S, Bidart J-M, Arturi F et al. Sodium/iodide symporter: a key transport system in thyroid cancer cell metabolism. *European Journal of Endocrinology* 1999; 141: 443–457.
- \* 10. Koong SS, Reynolds JC, Movius EG et al. Lithium as a potential adjuvant to  $^{131}\text{I}$  therapy of metastatic, well differentiated thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1999; 84: 912–916.
11. Tanaka K, Otsuki T, Sonoo H et al. Semi-quantitative comparison of the differentiation markers and sodium iodide symporter messenger ribonucleic acids in papillary thyroid carcinomas using RT-PCR. *European Journal of Endocrinology* 2000; 142: 340–346.
- \* 12. Pineda JD, Lee T, Ain KB, Reynolds JC & Robbins J. Iodine- $^{131}\text{I}$  therapy for thyroid cancer patients with elevated thyroglobulin and negative diagnostic scan. *Journal of Clinical Endocrinology and Metabolism* 1995; 80: 1488–1492.
13. Ringel MD, Ladenson PW & Levine MA. Molecular diagnosis of residual and recurrent thyroid cancer by amplification of thyroglobulin messenger ribonucleic acid in peripheral blood. *Journal of Clinical Endocrinology and Metabolism* 1998; 83: 4435–4442.
14. Boghaert ER, Ain KB, Taylor K et al. Quantitative and qualitative differences in growth, invasion, and metastasis of an anaplastic and papillary human thyroid cancer cell line in vitro and in vivo. *Journal of Clinical and Experimental Metastases* 1996; 14: 440–450.
15. Brabant G, Hoang-Vu C, Cetin Y et al. E-cadherin: a differentiation marker in thyroid malignancies. *Cancer Research* 1993; 53: 4987–4993.
16. Graff JR, Greenberg VE, Herman JG et al. Distinct patterns of E-cadherin CpG island methylation in papillary, follicular, Hurthle's cell, and poorly differentiated thyroid carcinoma. *Cancer Research* 1998; 58: 2063–2066.
17. Husmark J, Heldin NE & Nilsson M. N-cadherin-mediated adhesion and aberrant catenin expression in anaplastic thyroid carcinoma cell lines. *International Journal of Cancer* 1999; 83: 692–699.
18. Carcangiu ML, Zampi G, Pupi A, Castagnoli A & Rosai J. Papillary carcinoma of the thyroid: a clinicopathologic study of 241 cases treated at the University of Florence, Italy. *Cancer* 1985; 55: 805–828.

nt of this cancer  
nniques, which are  
of life and prolong

cer Institute of the  
ns Affairs.

id carcinoma with the  
idology, pp 1317-1321.

gnostic determinant in  
6; 81: 4318-4323.  
uman thyroid cell lines.

thyrotropin receptor  
H-R. *British Journal of*

symporter in human

activity is present in  
id cells. *Proceedings of*

perimental  $^{131}\text{I}$  therapy in  
ter gene. *Endocrinology*

id peroxidase in benign

stem in thyroid cancer

therapy of metastatic.  
ism 1999; 84: 912-916.  
entiation markers and  
inomas using RT-PCR.

id cancer patients with  
y and *Metabolism* 1995;

rent thyroid cancer by  
od. *Journal of Clinical*

growth, invasion, and  
and in vivo. *Journal of*

thyroid malignancies.

G island methylation in  
ancer Research 1998; 58:

catenin expression in  
72-699.

oma of the thyroid: a  
ancer 1985; 55: 805-828.

19. Kurozumi K, Nakao K, Nishida T et al. Significance of biologic aggressiveness and proliferating activity in papillary thyroid carcinoma. *World Journal of Surgery* 1998; 22: 1237-1242.
20. Nishida T, Kazayama S, Masahiko T, Nakamura J & Matsuda H. Clinicopathological significance of poorly differentiated thyroid cancer. *American Journal of Surgical Pathology* 1999; 23: 205-211.
21. Yamashita H, Noguchi S, Murakami N, Kawamoto H & Watanabe S. Extracapsular invasion of lymph node metastasis is an indicator of distant metastasis and poor prognosis in patients with thyroid papillary carcinoma. *Cancer* 1997; 80: 2268-2272.
22. Bätge B, Dralle H, Padberg B, von Herbay B & Schröder S. Histology and immunocytochemistry of differentiated thyroid carcinomas do not predict radioiodine uptake: a clinicomorphological study of 62 recurrent or metastatic tumours. *Virchows Archiv A Pathological Anatomy and Histology* 1992; 421: 521-526.
23. Vassilopoulou-Sellin R, Schultz PN & Haynie TP. Clinical outcome of patients with papillary thyroid carcinoma who have recurrence after initial radioactive iodine therapy. *Cancer* 1996; 78(3): 493-501.
24. Ain KB. Papillary thyroid carcinoma: etiology, assessment, and therapy. *Endocrinology and Metabolism Clinics of North America* 1995; 24: 711-760.
25. Venkataraman GM, Yatin M, Marcinek R & Ain KB. Restoration of iodide uptake in dedifferentiated thyroid carcinoma: relationship to human  $\text{Na}^+/\text{I}^-$  symporter gene methylation status. *Journal of Clinical Endocrinology and Metabolism* 1999; 84: 2449-2457.
26. Sobrinho-Simões M, Nesland JM & Johannessen JV. Columnar-cell carcinoma. Another variant of poorly differentiated carcinoma of the thyroid. *American Journal of Clinical Pathology* 1988; 89: 264-267.
27. Herrera MF, Hay ID, Wu PS-C et al. Hürthle cell (oxyphilic) papillary thyroid carcinoma: a variant with more aggressive biologic behavior. *World Journal of Surgery* 1992; 16: 669-675.
28. Papotti M, Torchio B, Grassi L, Favero A & Bussolati G. Poorly differentiated oxyphilic (Hürthle cell) carcinomas of the thyroid. *American Journal of Surgical Pathology* 1996; 20: 686-694.
29. Albareda M, Puig-Domingo M, Wengrowicz S et al. Clinical forms of presentation and evolution of diffuse sclerosing variant papillary carcinoma and insular variant of follicular carcinoma of the thyroid. *Thyroid* 1998; 8: 385-391.
30. Ozata M, Suzuki S, Miyamoto T et al. Serum thyroglobulin in the follow-up of patients with treated differentiated thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 1994; 79: 98-105.
31. Clark OH, Siperstein AE, Miller R & Claassen HR. Receptors and receptor-transducing systems in normal and neoplastic human thyroid tissue. In Goretzki PE & Röher HD (eds) *Growth Regulation of Thyroid Gland and Thyroid Tumors*, pp 14-27. Basel: Karger, 1989.
32. Derwahl M, Broecker M & Kraiem Z. Thyrotropin may not be the dominant growth factor in benign and malignant thyroid tumors. *Journal of Clinical Endocrinology and Metabolism* 1999; 84: 829-834.
33. Ain KB. Anaplastic thyroid carcinoma: behavior, biology, and therapeutic approaches. *Thyroid* 1998; 8: 715-726.
34. Venkataraman GM, Yatin M & Ain KB. Cloning of the human sodium-iodide symporter promoter and characterization in a differentiated human thyroid cell line, KAT-50. *Thyroid* 1998; 8: 63-69.
35. Ain KB, Tofiq S & Taylor KD. Antineoplastic activity of taxol against human anaplastic thyroid carcinoma cell lines in vitro and in vivo. *Journal of Clinical Endocrinology and Metabolism* 1996; 81: 3650-3653.
36. Ain KB, Egorin MJ & DeSimone PA. Treatment of anaplastic thyroid carcinoma with paclitaxel: phase II trial using 96-h infusion. *Thyroid* 2000; 10: 587-594.
37. Space VL, Morris JS, Nichols TA et al. Longitudinal study of iodine in toenails following IV administration of an iodine-containing contrast agent. *Journal of Radioanalytical Nuclear Chemistry* 1998; 236: 71-76.
38. Lakshmanan M, Schaffer A, Robbins J, Reynolds J & Norton J. A simplified low iodine diet in  $^{131}\text{I}$  scanning and therapy of thyroid cancer. *Clinical Nuclear Medicine* 1988; 13: 866-868.
39. Reynolds JC. Percent  $^{131}\text{I}$  uptake and post-therapy  $^{131}\text{I}$  scans: their role in the management of thyroid cancer. *Thyroid* 1997; 7: 281-284.
40. Schlumberger M, Mancusi F, Baudin E & Pacini F.  $^{131}\text{I}$  therapy for elevated thyroglobulin levels. *Thyroid* 1997; 7: 273-276.
41. Goldman JM, Line BR, Aamodt RL & Robbins J. Influence of triiodothyronine withdrawal time on  $^{131}\text{I}$  uptake postthyroidectomy for thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 1980; 50: 734-739.
42. Chung J-K, Young S, Lee JS et al. Value of FDG PET in papillary thyroid carcinoma with negative  $^{131}\text{I}$  whole-body scan. *Journal of Nuclear Medicine* 1999; 40: 986-992.
43. Vassilopoulou-Sellin R, Palmer L, Taylor S & Cooksley CS. Incidence of breast carcinoma in women with thyroid carcinoma. *Cancer* 1999; 85: 696-705.
44. Esik O, Németh G & Eller J. Prophylactic external irradiation in differentiated thyroid cancer: a retrospective study over a 30-year observation period. *Oncology* 1994; 51: 372-379.
45. O'Connell MEA, A'Hern RP & Harmer CL. Results of external beam radiotherapy in differentiated thyroid carcinoma: a retrospective study from the Royal Marsden Hospital. *European Journal of Cancer* 1994; 30A: 733-739.

46. Nomori H, Horio H, Mimura T & Morinaga S. Massive hemoptysis from an endobronchial metastasis of thyroid papillary carcinoma. *Thoracic and Cardiovascular Surgery* 1997; 45: 205-207.
47. Smith SA, Hay ID, Goellner JR, Ryan JJ & McConahey WM. Mortality from papillary thyroid carcinoma: a case-control study of 56 lethal cases. *Cancer* 1988; 62: 1381-1388.
48. Smit JWA, Vielvoye GJ & Goslings BM. Embolization for vertebral metastases of follicular thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2000; 85: 989-994.
49. Brown JM & Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Research* 1998; 58: 1408-1416.
50. Graeber TG, Osmanian C, Jacks T et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 1996; 379: 88-91.
51. Hoskin PJ & Harmer C. Chemotherapy for thyroid cancer. *Radiotherapy and Oncology* 1987; 10: 187-194.
52. Casara D, Rubello D, Saladini G et al. Different features of pulmonary metastases in differentiated thyroid cancer: natural history and multivariate statistical analysis of prognostic variables. *Journal of Nuclear Medicine* 1993; 34: 1626-1631.
53. Scherübl H, Raue F & Ziegler R. Combination chemotherapy of advanced medullary and differentiated thyroid cancer. Phase II study. *Journal of Cancer Research and Clinical Oncology* 1990; 116: 21-23.
54. Schlumberger M & Parmentier C. Phase II evaluation of mitoxantrone in advanced non anaplastic thyroid cancer. *Bulletin of Cancer* 1989; 76: 403-406.
55. Samonigg H, Hossfeld DK, Spehn J, Fill H & Leb G. Actarubicin in advanced thyroid cancer: a phase I study. *European Journal of Cancer and Clinical Oncology* 1988; 24: 1271-1275.
56. Maheshwari YK, Hill CS Jr, Haynie TP III, Hickey RC & Samaan NA. <sup>131</sup>I therapy in differentiated thyroid cancer: M. D. Anderson Hospital experience. *Cancer* 1981; 47: 664-671.
57. Satake S, Sugawara I, Watanabe M & Takami H. Lack of a point mutation of human DNA topoisomerase II in multidrug-resistant anaplastic thyroid carcinoma cell lines. *Cancer Letters* 1997; 116: 33-39.
58. Loe DW, Deeley RG & Cole SPC. Biology of the multidrug resistance-associated protein, MRP. *European Journal of Cancer* 1996; 32A: 945-957.
59. Lehnert M. Clinical multidrug resistance in cancer: a multifactorial problem. *European Journal of Cancer* 1996; 32A: 912-920.
60. Venkataraman GM, Yatin M & Ain KB. MDR1 and MRP1 mRNA expression in primary thyroid cancers and cell lines. *79th Annual Meeting of the Endocrine Society*, 1997. Minneapolis, MN. p 248, 1997.
61. Hama Y, Shimizu T, Hosaka S, Sugeno A & Usuda N. Therapeutic efficacy of the angiogenesis inhibitor O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470) for human anaplastic thyroid carcinoma in nude mice. *Experimental Toxicology and Pathology* 1997; 49: 239-247.
62. Schmutzler C & Köhrle J. Retinoic acid redifferentiation therapy for thyroid cancer. *Thyroid* 2000; 10: 393-406.
63. Aria M, Tsumishima T, Isozaki O et al. Effects of retinoids on iodine metabolism, thyroid peroxidase gene expression, and deoxyribonucleic acid synthesis in porcine thyroid cells in culture. *Endocrinology* 1991; 129: 2827-2833.
64. del Senno L, Rossi R, Franceschetti P & degli Uberti EC. Expression of all-trans-retinoic acid receptor RNA in human thyroid cells. *Biochemistry and Molecular Biology International* 1994; 33: 1107-1115.
65. Namba H, Yamashita S, Morita S et al. Retinoic acid inhibits human thyroid peroxidase and thyroglobulin gene expression in cultured human thyrocytes. *Journal of Endocrinological Investigation* 1993; 16: 87-93.
66. Schmutzler C, Winzer R, Meissner-Weigl J & Köhrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochemistry and Biophysics Research Communication* 1997; 240: 832-838.
67. Venkataraman GM, Yatin M, Marcinek R & Ain KB. Inhibition of polyamine synthesis restores sodium/iodide symporter gene expression and activity in dedifferentiated thyroid carcinoma cell lines. *72nd Annual Meeting of the American Thyroid Association*, 1999. Palm Beach, FL, 1999.
68. Dressel U, Renkawitz R & Banihmad A. Promoter specific sensitivity to inhibition of histone deacetylases: implications for hormonal gene control, cellular differentiation and cancer. *AntiCancer Research* 2000; 20: 1017-1022.
69. Boland A, Ricard M, Opolon P et al. Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. *Cancer Research* 2000; 60: 3484-3492.
70. Cho JY, Xing S, Liu X et al. Expression and activity of human Na<sup>+</sup>/I<sup>-</sup> symporter in human glioma cells by adenovirus-mediated gene delivery. *Gene Therapy* 2000; 7: 740-749.
71. Mandell RB, Mandell LZ & Link CR Jr. Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Research* 1999; 59: 661-668.
72. Bilbao G, Gomez-Navarro J & Curiel DT. Targeted adenoviral vectors for cancer gene therapy. *Advances in Experimental Medicine and Biology* 1998; 451: 365-374.

- endobronchial metastasis of papillary thyroid carcinoma: metastases of follicular thyroid cancer (and problems) for cancer therapy with diminished apoptotic potential. *Oncology* 1987; 10: 187-194.
- metastases in differentiated thyroid cancer: prognostic variables. *Journal of Clinical Investigation* 1990; 116: 21-23.
- medullary and differentiated thyroid cancer: a phase II study in differentiated thyroid cancer. *Thyroid* 2000; 10: 105-115.
- human DNA topoisomerase II $\alpha$  protein, MRP. *European Journal of Cancer* 1997; 33: 33-39.
- in primary thyroid cancers. *Journal of Clinical Endocrinology and Metabolism* 1994; 78: 1097-1102.
- of the angiogenesis inhibitor endostatin in human glioma cells. *Journal of Clinical Investigation* 1993; 91: 179-184.
- thyroid cancer. *Thyroid* 2000; 10: 105-115.
- thyroid peroxidase gene expression. *Endocrinology* 1991; 128: 3484-3492.
- trans-retinoic acid receptor. *Journal of Clinical Endocrinology and Metabolism* 1994; 78: 1097-1102.
- thyroid peroxidase and sodium/iodide symporter. *Journal of Clinical Endocrinology and Metabolism* 1993; 77: 1857-1862.
- sodium/iodide symporter of functional symporter in thyroid cancer cell lines. *Journal of Clinical Endocrinology and Metabolism* 1997; 75: 391-395.
- synthesis restores sodium/iodide symporter in thyroid cancer cell lines. *Journal of Clinical Endocrinology and Metabolism* 1994; 78: 1097-1102.
- to inhibition of histone synthesis. *AntiCancer Research* 1997; 17: 3484-3492.
- thyroid sodium/iodide symporter in human glioma cells. *Journal of Clinical Endocrinology and Metabolism* 1997; 75: 391-395.
- using the sodium/iodide symporter gene therapy. *Advances in Cancer Research* 1999; 213: 213-214.
- Nagayama Y, Nishihara E, Iizuka M et al. Enhanced efficacy of transcriptionally targeted suicide gene/prodrug therapy for thyroid carcinoma with the Cre-loxP system. *Cancer Research* 1999; 59: 3049-3052.
- Spitzweg CS, Zhang S, Bergert ER et al. Prostate-specific antigen (PSA) promoter-driven androgen-inducible expression of sodium iodide symporter in prostate cancer cell lines. *Cancer Research* 1999; 59: 2136-2141.
- Fagin JA, Matsuo K, Karmakar A et al. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *Journal of Clinical Investigation* 1993; 91: 179-184.
- Blagosklonny MV, Giannakakou P, Wojtowicz M et al. Effects of p53-expressing adenovirus on the chemosensitivity and differentiation of anaplastic thyroid cancer cells. *Journal of Clinical Endocrinology and Metabolism* 1998; 83: 2516-2522.
- Narimatsu M, Nagayama Y, Akino K et al. Therapeutic usefulness of wild-type p53 gene introduction in a p53-null anaplastic thyroid carcinoma cell line. *Journal of Clinical Endocrinology and Metabolism* 1998; 83: 3668-3672.
- Fagin JA, Tang SH, Zeki K et al. Reexpression of thyroid peroxidase in a derivative of an undifferentiated thyroid carcinoma cell line by introduction of wild-type p53. *Cancer Research* 1996; 56: 765-771.
- Moretti F, Farsetti A, Soddu S et al. p53 re-expression inhibits proliferation and restores differentiation of human thyroid anaplastic carcinoma cells. *Oncogene* 1997; 14: 729-740.
- Zeki K, Tanaka Y, Morimoto I et al. Induction of expression of MHC-class-II antigen on human thyroid carcinoma by wild-type p53. *International Journal of Cancer* 1998; 75: 391-395.
- Foster BA, Coffey HA, Morin MJ & Rastinejad F. Pharmacologic rescue of mutant p53 conformation and function. *Science* 1999; 286: 2507-2510.
- Watanabe T & Sullenger BA. Induction of wild-type p53 activity in human cancer cells by ribozymes that repair mutant p53 transcripts. *Proceedings of the National Academy of Sciences of the USA* 2000; 97: 8490-8494.
- Ain KB & Taylor KD. Somatostatin analogs affect proliferation of human thyroid carcinoma cell lines in vitro. *Journal of Clinical Endocrinology and Metabolism* 1994; 78: 1097-1102.
- Ain KB, Taylor KD, Tofiq S & Venkataraman G. Somatostatin receptor subtype expression in human thyroid and thyroid carcinoma cell lines. *Journal of Clinical Endocrinology and Metabolism* 1997; 82: 1857-1862.
- Zlock DW, Greenspan FS, Clark OH & Higgins CB. Octreotide therapy in advanced thyroid cancer. *Thyroid* 1994; 4: 427-431.



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**